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STUDIES ON THE PHYSIOLOGIC EFFECTS OF FEVER TEMPERATURES*

THE THERMAL DEATH TIME OF NEISSERIA GONORRHEAE IN VITRO WITH SPECIAL REFERENCE TO FEVER TEMPERATURES

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THE ever changing therapy for disease caused by Neisseria gonorrheae is evidence of the unsatisfactory results obtained in general by clinicians. Chemotherapy and the use of specific products of the gonococcus are employed most frequently, although the use of local or general applications of heat by means of hot water or by diathermy is recommended by many physicians. There are those who induce fever in such patients by injecting foreign proteins, especially sterile milk and typhoid vaccines. The literature contains many favorable and unfavorable reports on such procedures. Much progress has been made recently in the application of diathermic and radiothermic fever therapy, and therefore this method is becoming more extensively used. The lack of uniformity of results from the use of these artificially induced fevers, when the thermolability of the gonococcus is considered, presents an important problem for further analysis. We believe that the determination of the thermal death time of infectious agents at fever temperatures is fundamental in establishing a basis for such therapy.

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HISTORY

Some interesting observations on the effect of fever temperatures on N. gonorrheae have been reported in the literature, although the investigators were not primarily concerned with the thermal death time of the organism. Finger, Ghon, and Schlagenhaufer[1] stated that they failed to produce an urethritis in man by injecting the gonococcus into the urethra of a patient with a fever of 39° or 40° C. However, they were always successful if the host was afebrile. Guiard[2] observed a patient with gonorrheal urethritis who was cured after an attack of scarlet fever. Neisser and Scholtz[3] stated that it was always more difficult to obtain a culture of the gonococcus from patients when they had a fever than when they were afebrile. Bogdan[4] described a case of gonorrheal urethritis in which the purulent discharge disappeared during an attack of pneumonia but returned after the fever had receded. Luys[5] noted a similar case, in which the urethral discharge subsided during an attack of mumps with a fever of 40° C. but returned after the patient was afebrile. Culver[6] described a case of gonorrheal urethritis that recovered after having malaria four days, in which there was a maximum temperature of 40.5° C. He further states that if the body temperature is suddenly elevated from normal to 39° C., the gonococcus is destroyed. Others have observed a similar disappearance of the purulent urethral discharge during a febrile reaction. We have also observed the absence of the purulent exudate in our male patients, and likewise in those women with a profuse vaginal discharge, after treatment with a fever of 41.5° C. for five hours, produced by the passage of high frequency currents through the trunk of the patient. However, during the fastigium of such fevers, there occurred a marked increase in the mucopurulent discharge from the lower birth canal, which subsided after the treatment. Boerner and Santos[7] heated the male urethra with "diathermy" currents, and were unable to isolate the gonococcus after ten hours at 39°, three hours at 41°, and fifty-seven minutes at 41.7° C. Ylppö[8] treated with hot-baths a five-year-old girl with vulvovaginitis. The bath at the beginning of the treatment was 39° C. and was gradually raised to 41.5° C. After a series of eight daily treatments for one hour he was unable to isolate the gonococcus.

In contrast to these observations, Nobl[9] reported a series of five cases that had, superimposed on a gonococcus infection, either pneumonia or pulmonary tuberculosis with a fever of 40° C. He states that the venereal disease was not influenced. Nicoll[10] likewise observed three cases of gonorrheal septicemia and arthritis immediately following an attack of scarlet fever with a marked febrile reaction.

Practically all bacteriologists consider that 37° C. is the most suitable temperature for the isolation and cultivation of N. gonorrheae, and that temperatures above 38° C. definitely injure the organism. Kiefer,[11] and Steinschneider and Schäffer[12] report that the gonococcus is killed in a few hours at a temperature of from 40° to 41° C. Wertheim[13] states that this organism grows well at a temperature of 40° C, and resists a temperature of 42° C.

However, this has not been confirmed by the observations of other investigators. Ungermann[14] observed several strains that resisted 41° C. for ten hours, while three or four strains survived 52° C. for seven hours. Santos[15] reported that he was able to isolate the gonococcus from pus if it was not kept longer than forty-five minutes at 45° C. or five minutes at 50° C. Koch and Cohn[16] believe that this microorganism is more resistant to fever temperatures in vivo than in vitro. They base their opinion upon the fact that the infection resists a temperature of 40° C. for several days in man, during the course of an acute infectious disease.

Many other observations have been made on the thermal death time of the gonococcus, but these are concerned principally with the length of time required to kill the organism when subjected to from 45° to 60° C., which are temperatures that cannot be tolerated by man.

METHOD

The following experiment was designed to establish a basis for the fever treatment of patients infected with N. gonorrheae. We desired to determine the length of time required by such temperatures as 39°, 40°, 41°, 41.5°, and 42° C. to destroy pure cultures of the gonococcus in vitro.

Fifteen strains were obtained for this study, eight of which (Nos. 1 to 8 inclusive) were sent to us through the courtesy of Dr. John Torrey of the Cornell Medical School, New York City. All were isolated in 1920 with the exception of No. 8, recovered in 1922, from cases of chronic urethritis in the male. These strains have been under artificial cultivation from ten to twelve years. During this period they have been kept on Torrey's semisolid, vitamine medium that he described in 1922.[17] We shall designate these strains as "old." Seven cultures (Nos. 9 to 15 inclusive) were isolated from one to four months previous to these studies by Mrs. Alice Leahy, of the Bacteriological Laboratories of Strong Memorial and Rochester Municipal Hospitals. The source of these cultures was variable—joint fluid, male urethral discharge, or cervical and vaginal discharges (see Table I). The recently isolated strains of gonococci are designated as "new." These cultures were isolated and kept on a glucose, ascitic blood agar, prepared as follows: To 100 c.c. of Douglas' agar[18] with a P_H of 7.5 to 7.6 was added 25 c.c. of ascitic fluid, 5 to 8 c.c. of defibrinated rabbits' blood, and 1 per cent glucose. Ten days before immersing the cultures in the water-baths to determine their thermal death time, all were transferred to a glucose, ascitic, fluid broth, prepared by adding to 100 c.c. of a meat extract broth 25 c.c. ascitic fluid and 1 per cent glucose. After two or three generations in this broth, a 100 c.c. flask of the same medium was inoculated with the various cultures and allowed to incubate at 37° C. for forty-eight hours. The cultures were thoroughly shaken to break up the granular colonies and to insure a uniform suspension.

With a 20 c.c. Luer syringe, amounts of 1½ c.c. of the culture were then transferred to small glass vials, sealed, labeled, tied to dental film holders, and immersed in a series of water-baths. The baths were set at temperatures of 37°, 39°, 40°, 41°, 41.5°, and 42° C. The variation of the temperature of

the baths is no greater than 0.002° C. A complete description of the baths used for this work is published elsewhere.[19] At hourly intervals for thirty hours, one vial was removed from each bath and unviability of the organism was tested in the following manner: After breaking the stem of the vial with a file the contents of the vial were removed with a sterile Wright pipette and placed in a sterile tube. One-half cubic centimeter was pipetted onto the slant of a tube of glucose, ascitic blood agar, and the stoppers sealed in with paraffin. One-tenth cubic centimeter was then added to 100 c.c. of a sterile, physiologic salt solution for plating. The medium used for plating was a

TABLE I

SOURCE AND DATE OF ISOLATION OF GONOCOCCUS CULTURES USED IN EXPERIMENT

NUMBER	SOURCE	SEX	DATE OF ISOLATION
1	Urethra	Male	1920
2	Urethra	Male	1920
3	Urethra	Male	1920
4	Urethra	Male	1920
5	Urethra	Male	1920
6	Urethra	Male	1920
7	Urethra	Male	1920
8	Urethra	Male	1922
9	Cervix	Female	1931
10	Joint	Male	1931
11	Joint	Male	1931
12	Urethra	Male	1931
13	Cervix	Female	1931
14	Joint	Female	1931
15	Cervix	Female	1931

glucose, ascitic fluid agar, prepared by adding 1 per cent glucose, 25 c.c. of ascitic fluid, and 5 c.c. of defibrinated rabbit's blood serum containing a few cells, to 100 c.c. of Douglas' agar with a P_H of 7.6. Dilutions of 1-1,000 and 1-10,000 were then made in the usual manner. As the thermal death time was approached in subsequent experiments, lower dilutions were used for plating and finally 0.10 c.c. of the culture to be examined for viability was plated directly. A slightly different medium was used for plating some of the cultures. This medium, which is a hemoglobin serum agar, and is extensively employed in England for the cultivation of N. gonorrheae, was prepared as follows: To 100 c.c. of liquefied, 2 per cent digest agar was added 15 c.c. of sterile horse serum and then 1 c.c. of laked horse blood cells. The serum and hemolyzed cells were added after the agar had cooled to a temperature of 50° C. or below. The red blood cells were laked with chloroform.

The cultures were incubated at 37° C. The plates were counted forty-eight hours after they were made, while the tubes were examined for growth in seventy-two hours. The tubes and plates served as an excellent check on each other. The tubes gave us a more favorable medium and better atmospheric conditions for growing the gonococcus, while making plate counts gave us an opportunity to study the rate the gonococci were destroyed.

The number of gonococci per cubic centimeter in the broth cultures at the time the experiment began varied from 1,200,000 to 25,000,000. We ob-

served that this variation in the number of organisms in the suspensions to be heated in the water-baths was not as important a factor as the age of the suspension. A forty-eight hour growth was found to be the most resistant under the conditions with which we worked.

The thermal death time of the cultures was checked in two ways. First, the same strain was used in all the baths, hourly examinations being made. With this method it was difficult to work with more than two or three different strains at one time. Later several strains were exposed to the temperature of only one bath and examined hourly. Determinations of the number of hours required to destroy the gonococci at the various temperatures were made of each strain at least three times.

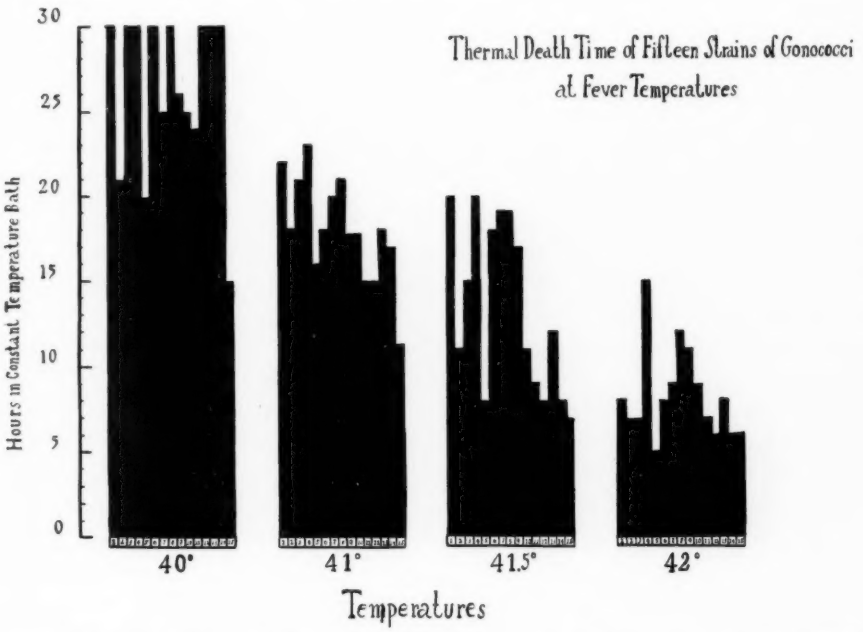

Fig. 1.—Showing the thermal death time of the fifteen strains of gonococci. Black lines represent death of all organisms.

RESULTS

The bath set at 37° C. was used for the control temperature. The vials containing these cultures were examined at hourly intervals for the length of time of the experiment, and, in general, the number of gonococci continued to increase throughout the thirty hour period. This indicated that there was sufficient food available for the organisms to grow and that atmospheric conditions and P_H values were satisfactory during the experimental period.

It was observed that 39° C. apparently had some injurious effect on the growth of the organisms because the counts showed practically no change in the original number of organisms in the suspension before exposure.

The results of the lethal effects of the fever temperatures 40°, 41°, 41.5°, and 42° C. are shown in Fig. 1. Inspection of these data gives three general

impressions. The first is that the thermal death time of the gonococcus at these fever temperatures is very variable. Second, the thermal death time of some of the strains is within the limits of fever temperatures that can be produced safely in man. Third, the cultures of gonococci that have been under cultivation for several years are, on the average, more resistant to fever temperatures than those recently isolated.

A temperature of 40° C. for thirty hours failed to destroy five (1, 3, 4, 6, and 8) of the eight "old" strains, while only three (12, 13, and 14) of the recently isolated strains of gonococci were still viable at the end of this period. The other three strains (2, 5, and 7) under cultivation for approximately twelve years were not viable after twenty-one, twenty, and twenty-five hours respectively. The other four recently isolated strains (9, 10, 11, and 15) required twenty-six, twenty-five, twenty-four, and sixteen hours respectively at 40° C. to obtain death of all of the organisms.

At 41° C., twenty-three hours was the longest period of time required to destroy all of the gonococci (strain 4 "old"). Eighteen hours was the maximum exposure at this temperature necessary to kill all cells in any of the cultures in cultivation for only a short period of time (strains 9, 10, 13 "new"). The shortest thermal death time for the "old" strains at 41° C. was sixteen hours (strain 5), while that of the "new" strains was eleven hours.

A fever of 41.5° C. for five hours artificially induced by high frequency currents or by short radio waves (30 meters) is a procedure that has been used in this clinic for the treatment of subacute and chronic gonorrhea. We had previously determined that this is the maximum temperature that can be employed for this length of time safely in fever therapy. Occasionally a patient's rectal temperature has registered 42° C. during the course of an artificial fever, but for only short intervals. In one or two instances the fever has been prolonged for seven hours, if the patient was in good physical condition at the end of five hours. Therefore, this is a critical temperature in these studies on the thermal death time of the gonococcus. At 41.5° C. a heating for twenty hours in the water-bath was necessary to obtain death of all the organisms in two "old" strains (1 and 4). Seventeen hours' exposure was required to kill a "new" strain,[9] which is only one hour less than that needed at 41° C. However, the other "new" strains were much less resistant at 41.5° C., and showed a shorter thermal death time, varying from seven to twelve hours at this temperature. The shortest exposure required to kill all organisms in the "old" strains was seven hours (strain 5). However, the majority were more resistant.

Strain 4 "old" was killed in fifteen hours at 42° C., but this culture was the most resistant of any of the "old" or "new" strains. The longest exposure at this temperature necessary to cause death of all the gonococci in the "new" series was eleven hours. However, six hours destroyed all the organisms in three of the "new" strains, which was the shortest thermal death time for this group at this temperature. Strain 5 "old" was killed in five hours. It will be noted that this is the least resistant strain of all of those under cultivation for twelve years. At all the fever temperatures its thermal

death time was similar to the more recently isolated strains. During the time we have studied this strain it has never grown so luxuriantly as the other "old" strains.

In the series of "new" cultures are two strains, 11 and 12, isolated respectively from a knee joint and from the urethra of the same patient. It is interesting to note that at 40° C. the urethral strain was more resistant than the joint strain, the former being still viable at thirty hours, while the latter was killed in twenty-four hours. At 41° C. the thermal death time was identical, while at 41.5° and 42° C. the joint strain was more resistant, requiring one hour more to obtain death of all the organisms than did the strain isolated from the urethra.

TABLE II

RESULTS OF PLATE COUNTS MADE ON GONOCOCCUS CULTURE NO. 15 AT FEVER TEMPERATURES

HOURS	37° C.	39° C.	40° C.	41° C.	41.5° C.	42° C.
0	3,100,000	2,400,000	3,800,000	3,400,000	2,120,000	4,780,000
1				2,190,000	258,000	312,000
2	2,200,000	6,100,000	400,000	220,000	640	24,000
3				56,000	720	120
4	3,330,000	contam.		2,000	580	176
5				6,000	84	9
6	1,168,000	2,090,000	360,000	1,600	14	
7				3,360	20	
8	2,650,000	3,030,000	760,000	380		
9				contam.		
10	2,400,000	1,100,000	11,000	412		
11				14		
12	2,520,000	4,570,000	26,000			
13						
14	4,160,000	5,200,000	11,000			
15						
16	3,420,000	720,000	42			
17						
18	6,120,000	3,800,000				
19						
20	8,600,000	1,230,000				

Studies of the plate counts made on several of the cultures used in this experiment indicate that at temperatures of 41.5° and 42° C., 87.8 per cent and 93.4 per cent respectively, of the gonococci were killed during the first hour (see Table II). At 41° C., 35.5 per cent were destroyed during the first hour, and 93.5 per cent by the end of the second hour. Four, two, and two hours' exposure at 41°, 41.5°, and 42° C. respectively, caused death of 99.9 per cent of the gonococci in the above culture (No. 15), which results are representative of the death curve of the other strains examined. At 40° C., 89.4 per cent died during two hours' exposure, while 95.2 per cent were dead at the end of six hours' exposure and 99.7 per cent were killed in ten hours.

DISCUSSION

The P_H of the broth cultures was determined when they were forty-eight hours old, at the time they were immersed in the water-baths, and after a period of thirty hours at the various temperatures. The P_H was determined by the colorimetric method, and Table III gives the results obtained on one

"new" and on one "old" strain. It is evident from this table that the change in P_H was not great enough to be an important factor in the cause of death of the gonococci. Inasmuch as the control cultures at 37° C. usually showed an increase in the number of microorganisms during the first fifteen or twenty hours of the experiment, evidently enough food was available in the glucose ascitic broth medium to support growth. Therefore, by eliminating a marked change in the P_H factor and by insuring a sufficient amount of food, the heat from the water-baths set at fever temperatures must have been responsible for their death.

At temperatures of 41°, 41.5°, and 42° C., autolysis of some of the gonococci occurred from one to two hours before death of all the organisms. This was indicated by a clearing of the medium, and also by making and gram staining smears from the culture at the thermal death time. Autolysis was not observed at 39° C., and was only slight after about twenty hours at 40° C. At the higher temperatures there was not always complete autolysis.

TABLE III

RESULTS OF DETERMINATION OF P_H OF FORTY-EIGHT HOUR CULTURE OF TWO STRAINS OF N. GONORRHEAE BEFORE AND TWENTY-FOUR HOURS AFTER EXPOSURE TO THE FEVER TEMPERATURES

STRAIN	TIME	37° C.	39° C.	40° C.	41° C.	41.5° C.	42° C.
6	P_H Before	7.8	7.8	7.8	7.8	7.8	7.8
	P_H 30 Hours	7.8	7.7	7.7	7.7	7.8	7.8
9	P_H Before	7.8	7.8	7.8	7.8	7.8	7.8
	P_H 30 Hours	7.6	7.6	7.6	7.7	7.6	7.8

The lethal effect of fever temperatures on N. gonorrheae in clinical cases is a controversial matter. Some observers have reported that during naturally occurring, or induced, fevers, symptoms of the disease have subsided, while others state that body temperatures above normal, 39° to 41° C., have not been of therapeutic value or have caused only a temporary cessation of inflammatory discharges. The variation of the resistance of different strains to heat may be the explanation for such conflicting reports. A study of temperature gradients in the normal human body demonstrated that the temperatures of the urethral meatus and anterior urethra are from 1° to 4° C. lower than the rectal temperature. We have observed that during a fever a similar gradient persists, unless the temperature of the environment about the penis is maintained at or above the level of the elevated body temperature.

Although not all the organisms were killed in five hours at 41.5° C., it is significant that 99.9 per cent of them were destroyed in the first two hours of exposure to this temperature. Since the virulence of N. gonorrheae is difficult to determine experimentally, we have no method to find out if the 0.1 per cent of the organisms remaining viable were still pathogenic. It is evident that in vivo some injury other than that due to heating occurs to the gonococcus, making it more susceptible to destruction by the normal defense mechanism of the body. Clinically we have observed "cures" re-

sulting from such a fever as 41.5° C. for five hours, when we were unable to obtain an in vitro thermal death time for a similar exposure.

Plate cultures made from the cervix of cases of chronic gonorrhea before, during, and after artificial fever therapy demonstrate the effect of a temperature of 41.5° C. for five hours on the gonococcus. Many colonies of gonococci were present in smear plate cultures immediately before the treatment, while after five hours exposure no colonies of the gonococcus could be found. This result may be due to the excessive secretion of the cervical glands during the treatment which washed the gonococci from the surface of the cervix. The cultures were made by inserting a sterile swab into the external os of the cervix and then streaking the exudate on blood glucose ascitic agar. Other types of bacteria, especially staphylococci, diphtheroids, and large, unidentified gram-positive rods, were not affected to any great degree by such fevers.

The variability of the resistance of the strains to the fever temperatures was anticipated, especially at the lower temperatures (40° to 41° C.), when a comparatively long exposure was required. At least three determinations were made on each strain and invariably the thermal death time was identical. In some instances a variation of one hour longer or shorter thermal death time was observed for the necessary increased exposure at 40° and 41° C. The greater resistance of the "old" strains may be explained upon their better adjustment to artificial cultivation. Many of the different determinations were made on the same culture at intervals of from three to four months. Since practically no change in the thermal death time was noted in such strains during this time, it is evident that the greater resistance of the cultures to the heating is acquired slowly and after a comparatively long period on artificial media. That the gonococcus is more resistant to fever temperatures in vivo than in vitro is doubted. The abnormal environment resulting from artificial cultivation cannot be more injurious to the gonococcus than the natural defensive agents of the body, other than fever.

SUMMARY AND CONCLUSIONS

1. The thermal death time of fifteen strains of N. gonorrheae was determined in vitro at fever temperatures of 30°, 40°, 41°, 41.5°, and 42° C. Seven strains had been under cultivation for twelve years and one strain for ten years, while the remaining seven strains were isolated from one to four months prior to beginning the experiment.

2. The resistance of the strains of gonococci examined to the fever temperatures was variable. The cultures that were isolated ten and twelve years ago had, on the average, a longer thermal death time than the recently isolated cultures.

3. At 39° C. there was little, if any, effect on the growth of the organism. At 40° C. about 99.7 per cent of the organisms were killed by ten hours' exposure, while death of all of the cells was not obtained at thirty hours in eight of the strains.

4. At 41° C., 99 per cent of the gonococci were destroyed in from four to five hours' exposure, while death of all the organisms required from eleven to twenty-three hours.

5. At 41.5° and 42° C., 99 per cent of the gonococci were rendered nonviable in two hours. The remainder were killed at 41.5° C. from seven to twenty hours, while at 42° C. the thermal death time varied from five to fifteen hours. In all instances the recently isolated cultures, with the exception of one "old" strain, showed the least resistance to 41°, 41.5°, and 42° C.

6. The in vitro thermal death time of N. gonorrheae is short enough at 41°, 41.5°, and 42° C. to suggest artificially induced fever as a valuable aid in the treatment of disease due to this organism. It is doubtful whether complete sterilization by heat can be obtained always from a single artificially induced fever of five hours' duration.

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BIOCHEMICAL STUDIES ON THE MECHANISM OF PHENYLCINCHONINIC INTOXICATION*

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I

APPLICATION OF A NEW FUNCTIONAL LIVER TEST TO PHENYLCINCHONINIC INTOXICATION IN ANIMALS

IN THE course of the last few years, a number of fatal cases of phenylcinchoninic intoxication in human beings have been observed (most of them after excessive doses or after unduly prolonged administration of the drug), manifesting themselves as yellow *atrophy of the liver*. It seemed therefore interesting to establish, whether it may be possible to detect by the chemical examination of urine some signs of liver injury in animals during phenylcinchoninic intoxication.

We recently published a paper[1] dealing with "aromaturia," sometimes depending on severe liver injury, and we, therefore, tried to profit from this new method for the above mentioned purpose. It is generally known, that the Millon test is characteristic for phenol, as well as, for phenolic substances (such as tyrosine), and that under pathologic conditions some Millon substances occasionally appear in the urine. We tried to study the conditions which may be responsible for this occurrence. The substances, yielding the Millon test, may be separated into two groups, so far as they are either soluble or insoluble in ether. It is easy to extract with ether certain acids derived from tyrosine from the urine and to estimate them colorimetrically. But we met with considerable difficulties when we tried to make quantitative researches on the bulk of the Millon substances in the urine, which are insoluble in ether. These phenolic substances belong to a group of certain high molecular and unidentified substances in the urine, which are derived from proteins and known as oxyproteic acids. Among these oxyproteic acids there are, occasionally, appreciable quantities of phenolic substances, which in this case are responsible for the Millon test of the urine.

Several years ago, one of us,[2] together with his students, evolved a method for the determination of the tyrosine content of proteins based on the Millon test. The idea of this method is, that from protein hydrolysates interfering substances are removed by phosphotungstic acid; the excess of this reagent, then, by quinine; and the excess of quinine by sodium hydrate. The phenolic substances, contained in the last colorless filtrate, may then be estimated colorimetrically by way of the Millon reaction, using a 0.1 per cent solution of tyrosine as a standard. Boiling is avoided; the color develops on standing at room temperature in the course of forty-five minutes.

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We found, that the most convenient way to remove all those urinary substances interfering with the Millon test,* is to precipitate the urine in the same manner as is usual in the determination of urea by the method of Mörner-Sjöquist.† The barium containing precipitate is separated and then disintegrated by 10 per cent H_2SO_4. $BaSO_4$ is removed by filtration and the filtrate then treated like a protein hydrolysate.‡ For the final colorimetry a Bioklett colorimeter was used.

We examined many urines by this method and we found that this method is a test for severe injuries of the liver (such as icterus gravis, yellow atrophy of the liver, phosphorus poisoning, etc.) and for increased desintegration of body proteins (for instance in advanced pulmonary consumption).

We took advantage of this new method, revealing severe disturbances of the liver, in order to establish, *whether an increased output of phenolic substances, contained in the oxyproteic fraction, may be observed in rabbits, poisoned by large doses of phenylcinchoninic acid (cinchophen) or the ester of paramethylphenylcinchoninic acid (tolysin).*[2a]

We performed experiments on seven rabbits: (a) subacute cinchophen poisoning, death after five weeks; (b) subacute tolysin poisoning, death after nineteen days; (c), (d), (f), (g) subacute tolysin poisoning, death after three or four days; (e) mild tolysin poisoning, survived.

The result of these experiments was negative. Only once was a temporary occurrence of a phenolic substance in the urine ascertained. We may conclude therefore, that *neither in 6 cases of acute or subacute tolysin poisoning in rabbits, nor in one case of subacute cinchophen poisoning did the liver seem to have been severely injured.* Perhaps it will be possible to get positive results on animals in cases of chronic intoxication, when prolonging the experiments to a duration of several months or using a more delicate liver test, enabling us to detect initial liver changes as possible precursors of severe liver injuries and of acute yellow atrophy. Besides we saw the appearance of Millon bodies (corresponding to the quantity of 15 to 30 mg. per cent) in the urine of a hyperthyroidized rabbit in a case of subacute cinchophen poisoning, not immediately, but only a month after the last injection of cinchophen (0.4 gm. p.k.). The animal was killed soon afterward. But careful examination of the liver (Professor Carl Sternberg) did not reveal any histologic changes.

II. EXPERIMENTS CONCERNING THE ALLEGED POSSIBILITY OF CINCHOPHEN DETOXICATION BY A DIET ABUNDANT IN CARBOHYDRATES

Many years ago one of us (Fürth[3]) observed, that rabbits, when overloaded with carbohydrates, tolerated larger doses of phosphorus, than rabbits, kept on a mixed diet.§

*The most important of these substances is *urea* on account of its interaction with nitrous acid, which is indispensable for the Millon test.

†Fifty c.c. of the urine are mixed with 15 gm. of powdered Ba $(OH)_2$ together with 1000 c.c. of a mixture containing two-thirds of alcohol and one-third of ether.

‡For further details we refer to the above cited paper.[1]

§So for instance a rabbit, that had received 0.005 gm. of yellow phosphorus, when fed with mixed food, died within a week, whereas another animal of nearly the same size, that had received the double quantity of phosphorus and, besides mixed food, 30 gm. of *glucose* daily by the pharyngeal sound, lived thirty days.

The fact, that carbohydrates exhibit a protective power against phosphorus poisoning, was recently confirmed in this laboratory by R. Scholl's[4] experiments on rats. The minimal lethal dose of phosphorus for albino rats, when given subcutaneously, is 8 mg. per kilogram body weight. The lethal dose is increased by a diet abundant in carbohydrates of 12 to 15 mg. per kilogram. As carbohydrates starch and inulin* (yielding levulose on hydrolysis) were used. In those rats, which because of carbohydrate feeding had survived a lethal dose of phosphorus, the liver was found to be rich in glycogen and poor in fat; just the contrary was true for rats, which had succumbed to the intoxication. (See also Arndt and Greiling.[5])

It is generally known, that phosphorus causes a fatty degeneration of the liver, rather similar to acute yellow atrophy of the liver. Several clinicians[6] claim, that they were able to save cases of icterus gravis (beginning yellow atrophy of the liver) by the combination of intravenous sugar injections with insulin.

It is therefore quite logical, that different attempts were made, to treat cases by abundant administration of carbohydrates, where patients were affected with more or less severe icterus in consequence of an intoxication caused by an abuse of phenylcinchoninic drugs.

We therefore tried to establish by four series of experiments on cinchophen-poisoned rats, whether it is possible to influence this intoxication favorably by glycogen storage in the liver.

We came to the conclusion, that the *minimal lethal dose of cinchophen for albino rats was nearly the same, whether the diet was rich in carbohydrates or entirely free from carbohydrates.* These experiments again seem to prove, that in acute cinchophen intoxication a liver injury, provided that there is any, certainly does not play a dominating part. It goes without saying, that this does not prove anything for *delayed phenylcinchoninic intoxication in human beings* and that, in spite of the negative outcome of these experiments, it seems reasonable to try an abundant administration of carbohydrates in certain cases, where there is a suspicion of a beginning icterus gravis in consequence of an abuse of phenylcinchoninic drugs.

III. PHENYLCINCHONINIC INTOXICATION INFLUENCED BY HYPERTHYROIDISM

There are several indications, which suggest the idea that hyperthyroidism might possibly be one of those unknown factors, which, under certain conditions, in individuals uncommonly sensitive for phenylcinchoninic drugs, may cause a kind of "idiosyncrasy." It is generally known that in experimental hyperthyroidism as well as in exophthalmic goiter there is a greatly increased sensitiveness of the sympathetic nervous system. We know on the other hand, that phenylcinchoninic drugs exhibit an analgesic, antipyretic and antirheumatic action by influencing certain nervous centers, which are in close correlation with the sympathetic nervous system.

*It has been established by Neubauer, that in phosphorus poisoning levulose is more readily stored in the liver, than glucose.

The following experiments seem indeed to show, that phenylcinchoninic intoxication in animals is markedly influenced by hyperthyroidism.

a. Eighteen *albino rats* were hyperthyroidized by thyroid feeding in the course of eight days. The normal lethal dose of cinchophen for rats is 0.55 to 0.65 gm. per kilogram. It was diminished hy hyperthyroidism (controlled by the progress of body weight) to 0.35 to 0.40 gm. p.k.

b. Three *guinea pigs* were hyperthyroidized in the course of sixteen days by 11 injections of thyroxin (¼ or ⅛ mg. each). Doses of cinchophen of 0.4 gm. per kilogram, corresponding to about ⅔ of the lethal dose (Fürth and Kuh[2a]) produced enormous falls of the rectal temperature to less than 34°.

c. Three *rabbits* were hyperthyroidized by 6 injections of thyroxin (¼ or ½ mg. thyroxin each) in the course of seven days. They died after having received 0.4 gm. p.k. cinchophen subcutaneously, i.e., about one-third of the normal lethal dose.

We may learn from the foregoing experiments that the *toxicity of cinchophen is decidedly increased by simultaneous hyperthyroidism.*

IV. DIFFUSION OF DYESTUFFS INTO GELATIN INFLUENCED BY CINCHOPHEN

The leading idea of our further investigations, bearing on the mechanism of phenylcinchoninic intoxication, was a *systematic study of colloidal changes induced by the phenylcinchoninic anion.* We began with the study of the diffusion of dyestuffs into gelatin as influenced by the presence of cinchophen.

K. Franke[7] at the medical clinic of Brugsch (Halle a/S) observed an increased passage of eosin sodium from serum into gelatin in presence of a small quantity of atophan, and he considered this phenomenon as analogous to the increased passage of eosin into bile owing to the ingestion of atophan into the living organism.

a. A hot 10 per cent solution of purest gelatin was poured into test tubes and allowed to solidify. A water solution of eosin 0.05 per cent, containing 0, 0.5, 1.0, 2.0 per cent of phenylcinchoninic acid (as sodium salt) was placed over the solidified jelly and the process of the diffusion of the dyestuff observed and measured.

EOSIN SOLUTION CONTAINING PHENYLCINCHONINIC ACID	PROGRESS OF THE DIFFUSION AFTER DAYS			
	1	2	3	4
per cent	measured in millimeters			
0	10	15	17	22
0.5	13	15	23	26
1.0	14	15	25	26
2.0	14	15	27	28

There was a *well marked increase in the path of diffusion (Diffusionsweg) caused by the presence of the phenylcinchoninic anion.*

b. The experiment was repeated with eosin (0.05 per cent) and neutral red (0.05 per cent) and varied in such a way, that the phenylcinchoninic anion was either contained in the color solution or in the solidified jelly itself. The reaction of the solution of cinchophen sodium was strictly controlled in view of its reaction and found to be practically neutral to litmus: The result was the same as in a.

c. Diffusion of sodium sulphoindigotate (0.0125 per cent solution in water) into 10 per cent gelatin. The dyestuff solution (a) without addition; (b) containing cinchophen sodium 1 per cent; (c) containing sodium glycocholate 1 per cent; and (d) containing sodium salicylate 1 per cent. The glycocholate was used as a type of a substance, exhibiting a high surface energy. The velocity of diffusion was markedly increased only by the presence of the phenylcinchoninic salt, but not by the salicylate, nor by the glycocholate: The path of diffusion (Diffusionsweg) measured after four days at room temperature was in (a) 10 mm.; (b) 16½ mm.; (c) 10½ mm.; and (d) 11½ mm.

CONCLUSION

Diffusion of certain dyestuffs (eosin, neutral-red, sodium sulphoindigotate) into gelatin is markedly increased by the presence of the phenylcinchoninic anion. This phenomenon does not seem to be due to changes in surface tension, but probably to colloidal changes in protein molecules.

V. SWELLING OF GELATIN, INFLUENCED BY PHENYLCINCHONINIC ANION*

A.

A 10 per cent gelatin solution was allowed to solidify in a crystallization dish. After cooling small cylinders of about 2 cm. height were punched with a cork-borer and weighed in small closed weighing bottles. Then the gelatin cylinders were allowed to stand for twenty hours in contact with either 0.9 per cent sodium chloride solution or with physiologic saline containing 10 per cent of sodium cinchoninate. The percentage increase in weight was established:

Physiologic saline as such:	1.8919 gm.	1.8000 gm.		1.3001 gm.
	1.3991	1.2697		0.9481
	0.4928	0.5303		0.3520
	+36%	+38%		+37%
=				
Physiologic saline containing cinchophen:	1.8964	2.0674	1.8003	1.8445
	1.2225	1.3408	1.1995	1.2228
	0.6739	0.7266	0.6008	0.6217
	+58%	+54%	+50 %	+51%

The percentage increase by swelling therefore was in the physiologic saline 36 to 38 per cent, in the physiologic saline containing in addition 10 per cent of cinchophen as sodium salt, 50 to 58 per cent, though the osmotic pressure of the latter, counteracting the swelling, was considerably increased by the presence of the salt of phenylcinchoninic acid.

B.

In a further experiment we used a method, evolved by a Japanese author (Tomita[8]), which has the advantage of great simplicity: A hot 10 per cent gelatin solution is allowed to solidify in a crystallization dish of about 12 cm. in diameter with flat bottom and cylindric walls. After cooling 30 c.c. of the fluid, which is to be examined, are poured in the crystallization dish, which then is closed by a cover, in order to avoid evaporation. From time to time the fluid is poured off in a graduated cylinder and measured. The decrease in

*A. v. Kuthy (Biochem. Ztschr. 237, 396, 1931) found quite recently in the laboratory of E. Verzar (Debreczen), that those salts, which belong to the type of hydrotropic substances (C. Neuberg), such as the sodium salts of benzoic acid, phenylactic acid, phenyl-propionic acid, benzolsulfonic acid, increase the swelling of gelatin and the passage of dyestuffs into gelatin. M. Popoff and K. Saisoff (Veterinary School, Berlin, Biochem. Ztschr. 156, 196, 1925) already found, that salicylic acid increases the intake of water by gelatin.

its volume, indicating the intake of fluid by the swelling gelatin, is a relative index of the intensity of swelling. We used (a) NaCl 0.9 per cent and (b) NaCl 0.9 per cent, containing 1 per cent of the sodium salt of cinchophen. The intake of fluid by the gelatin was also in this case markedly increased by the presence of the phenylcinchoninic salt.

C.

We performed several experiments in which, instead of gelatin, the swelling of frogs' muscles was studied. We used both the excised musculi gastrocnemii of a decapitated frog for each experiment and control, removing the adherent fluid carefully by hardened filter paper and weighing them accurately in closed wide mouthed weighing bottles. But we could not establish a marked difference caused by the presence of the phenylcinchoninic anion. This failure may be due to the gradual development of lactic acid from lactacidogen within the muscle. As lactic acid greatly increases the swelling of proteins, the effect of the phenylcinchoninic anion on swelling seems to have been masked by the superposition of this dominating factor.

CONCLUSION

The swelling of gelatin in 0.9 per cent sodium chloride solution is markedly increased by the presence of the phenylcinchoninic anion.

VI. THE SPONTANEOUS COAGULATION OF TISSUE PROTEIN SOLUTIONS INCREASED BY PHENYLCINCHONINIC AND SALICYLIC ANION

More than thirty-five years ago one of us (Fürth[9]) based on the earlier experiments of W. Kühne (1864) and W. D. Halliburton (1888) studied in F. Hofmeister's laboratory the spontaneous coagulation of the muscle proteins myosin and myogen and he thought that it proceeds according to the scheme:

Myosin (coagul. point 46-51°)	Myogen (coagul. point 55-65°)
↓	↓
	soluble Myogenfibrin (coagul.
	↓ point 30-40°)
Myosinfibrin	Myogenfibrin

In further researches, bearing on the interaction of certain poisons on the muscle proteins and their alleged influence on muscular rigidity[10] he observed, that some of these poisons greatly increased the spontaneous coagulation of myosin and myogen. The fact, that several of these drugs (sodium salicylate, antipyrin, quinine and cinchonin) are known as antipyretic and analgesic agents, gave us the idea to study the *influence of the phenylcinchoninic anion on the spontaneous coagulation of tissue proteins.*

A. Muscle Plasma.—Muscle plasma was prepared by suspending ¼ kilogram of hashed bovine muscles in ½ liter of physiologic saline and allowed to stand overnight: To samples of the filtered plasma were added equal parts of (a) NaCl 5 per cent, (b) cinchophen sodium 5 per cent and (c) sodium salicylate 5 per cent: (a) remained unchanged for more than one day and it was not before the second day, that a precipitate settled; (b) remained unchanged for one hour; after two hours there was a dense turbidity, which slowly after three days settled as a rich precipitate; (c) in the salicylate-containing samples there was a dense turbidity already after fifteen minutes and a rich precipitate settled in the course of the first day.

Myosin and Myogen were separated from the bulk of the muscle plasma, the first by addition of 1¼ volume of saturated ammonium sulphate solution, the second by complete saturation with the same salt. The myosin and myogen precipitates were redissolved in water and the solutions of the proteins treated in the same manner as described for muscle plasma a, b, c Myosin: (a) remained clear for one day; (b) the cinchophen test began to become turbid after two hours; (c) contained an opaque turbidity already after one hour. Myogen: (a) was unchanged after one hour and after one day there was only a poor precipitate; (b) and (c) settled to bulky precipitates already in the course of one hour.

There was no doubt, *that the spontaneous coagulation of muscle plasma and its protein constituents is greatly increased by phenylcinchoninic acid.*

B. *Liver Plasma.*—In quite the same manner a "plasma" was prepared from hashed calfs-liver. The result was analogous.

C. *Minimal Effective Quantity of Cinchophen.*—a. Myogen solution, added with the equal bulk of cinchophen sodium solution, varying in their concentrations from 5 per cent down to 0.01 per cent. After five hours the samples, containing 5 to 0.2 per cent cinchophen, were decidedly turbid, the 0.1 to 0.01 per cent samples remained unaltered.

b. Muscle plasma, *comparison of the efficiency of the phenylcinchoninic and salicylic anion;* dilutions varying between 5 and 0.01 per cent.

Cinchophen-Na-series: After one day the samples containing 1.5 to 0.3 per cent showed increased turbidity. The samples 5 to 2 per cent no turbidity. There was a reverse action, the precipitates being redissolved in an excess of the precipitant.

Sodium salicylate series: After one day there was a dense turbidity in the samples with 5 to 1½ per cent, slight turbidity in the samples with 1 per cent and 0.5 per cent. The samples with 0.4 to 0.01 per cent showed no more turbidity than the control samples without salicylate.

c. Liver Plasma.—Series of 15 samples, containing 2.5 to 0.005 per cent cinchophen sodium. There was an immediate, voluminous precipitation in the samples with 2.5 to 0.5 per cent. There was an immediate turbidity in the samples with 0.25 and 0.20 per cent. In the sample with 0.15 per cent cinchophen there was an opacity after half an hour. The series were placed in the incubator (40°) for three hours; whereas the control samples, containing no cinchophen, remained unchanged, there were decreasing precipitates in all the samples, even in those samples, which only contained 0.010 and 0.005 per cent of cinchophen.

D. *Buffer Solution Series With the Sodium Salts of Phenylcinchoninic and Salicylic Acid.*—It seemed to us important, to establish, how far the spontaneous coagulation of tissue proteins by the phenylcinchoninic and salicylic anion is influenced by the acidity of the medium. It is generally known, that the coagulation of proteins is increased mostly by increasing the acidity of the substrate. A series of phosphate buffer solutions was prepared according to P. Rona's[11] prescription by mixing m/15 solutions of KH_2PO_4 and $Na_2HPO_4.2H_2O$ in adequate proportions. The P_H of the resulting buffer solution was adequate to: (alkaline end) 8.3 to 4.5 (acid end of series).

It became evident, that the spontaneous coagulation of muscle plasma is greatly increased by the presence of both the phenylcinchoninic and the salicylic anion and that this effect increases with the acidity of the medium.

E. *Situation of the phenylcinchoninic and salicylic anion* in the Hofmeister series.*—It is known, that the efficiency of neutral salts in precipitating (salting out) proteins is an additive function of their anions and kations, which,

*The fact that salicylates stand beyond the rhodanid end of the Hofmeister series was discovered by R. Willheim (Kolloid-Ztschr. 48: 230, 1929).

according to this quality, may be arranged into series (Hofmeister's series). R. Höber and other authors have shown, that those anions, which in an acid medium are the most efficient ones, are the least efficient in an alkaline medium. Those anions, such as the iodide anion and the sulphocyanide anion, which greatly increase the precipitation (dehydration) of proteins in an acid medium, produce the contrary effect (increased swelling and solubility) in an alkaline medium. Hofmeister's anionic series (the succession of anions in an acid medium is mostly but not quite uniformly registered as CNS > I > NO_3 > Br > Cl > Acetate > SO_4 > Tartrate > Citrate > F) is widely responsible for many physiologic and chemical phenomena in their dependence on the presence of certain anions.* On account of the physiologic and pharmacologic importance of these facts it seemed interesting to ascertain, what place in the Hofmeister series may be due to the phenylcinchoninic and salicylic anions.

We may conclude from our experiments on muscle plasma and buffered solutions of muscle proteins, that the phenylcinchoninic as well as the salicylic anion are to be placed beyond the end of Hofmeister's anionic series:

$$\left.\begin{matrix}\text{Phenylcinchoninic anion}\\ \text{Salicylic acid}\end{matrix}\right\} > CNS' > I' > NO_3' \quad \ldots\ldots$$

They increase the spontaneous coagulation (dehydration) of tissue proteins more intensively even than the sulphocyanide and iodide anion, provided that the reaction is slightly acid. The artificial solutions of tissue proteins are always slightly acid, owing to the presence of lactic acid and they on the contrary will induce swelling (increased hydration) of tissue proteins, provided that the reaction is neutral or slightly alkaline.

We therefore may possibly understand the mechanism of the lesion of liver cells by phenylcinchoninic drugs from the point of view of colloidal chemistry: There may be increased swelling of the cells in the state of normal neutrality. No doubt that the increased swelling beyond a certain limit can injure the normal cell functions. It does not seem very probable that under the conditions of life the liver cells may ever become decidedly acid. But if, owing to any pathologic incidence, this might really happen, the presence of the phenylcinchoninic anion could possibly induce increased aggregation of protein particles, coagulation and necrosis.†

VII. INCREASED SPONTANEOUS COAGULATION OF PROTEIN SOLUTIONS FROM NERVOUS TISSUES BY PHENYLCINCHONINIC SALTS AND BY OTHER ANTIPYRETICS

The proteins of nervous tissues were first accurately studied by W. D. Halliburton.[16]

We prepared an extract from 250 gm. of finely minced calfs' brain with 500 c.c. of physiologic saline, allowing the suspension to stand overnight and

*For instance: precipitation of proteins and of lecithin, swelling of gelatin and muscles, hemolysis, irritability of muscle and nerves, electric currents arising from muscles and nerves, intensity of ciliary motion, liberation of phosphoric acid from lactacidogen, etc. (Bechhold,[12] Höber,[13] Embden and Lehnartz[14]).

†The dominating importance of the reaction of living tissues becomes evident from the experiments of Fürth and Schwarz[15] on working musculi gastrocnemii of living cats, left in situ: Whereas sodium salicylate and sodium sulphocyanide induce clotting of muscle plasma extra corpus, they greatly increase the energy production of the living working muscles.

centrifuging the strained turbid solution. This solution was saturated with ammonium sulphate, the precipitate of proteins collected on a folded filter paper, redissolved in about 20 c.c. of water and filtered again. Samples of ½ c.c. of the resulting clear, yellow solution were transferred into small test tubes and added with the equal bulk of m/15 sodium benzoate, sodium salicylate, sodium salt of cinchophen, sodium benzoate of caffeine, hydrochlorate of quinine, antipyrine and m/10 solution of hydrochlorate of morphia. After half an hour precipitates had settled in the cinchophen and quinine* tests, and there was a slight turbidity in the salicylate test; the other tests were unchanged. The next day the benzoate, caffeine, and morphia tests were still unaltered; there were opaque turbidities in the salicylate test and antipyrine test. This experiment was repeated with the addition of phosphate buffer solutions.

We performed numerous series of experiments of the same type and came to the following conclusion: Six substances, used as antipyretics, analgesics, and antirheumatics (phenylcinchoninic and salicylic anion, quinine, antipyrine, phenocoll and melubrin† were found to accelerate the spontaneous coagulation of solutions of the proteins of nervous tissues, of the muscle and the liver. The first two agents appeared to be superior to the other substances in this respect. It will require numerous further experiments to show the efficiency or nonefficiency of other antipyretics and to establish definitely the sequence and order of their relative quantitative efficiency from this special point of view, which may of course be greatly altered by accidental factors and by slight changes in the true acidity of the solutions (P_H). But the foregoing experiments seem sufficient for making it probable, that some changes in the colloidal state of the proteins of certain nervous centers (increased or decreased swelling) may play a part in the pharmacologic and physiologic behavior of these and related substances. As the reaction in the living tissues is considered to be neutral or slightly alkaline, we should rather believe in an increased than in a decreased hydration, manifesting itself in the slightly acid medium of tissue extracts.

It was only recently that we learned to know the new important investigations of Bancroft[19] and his associates (Rutzler, Richter) at Cornell University on the relation of swelling of cerebral colloids to narcosis and the action of analgesic drugs. Sodium thiocyanate, which was chosen because it peptizes protein strongly and stands at the head of the lyotropic series of anions, was supposed to antagonize morphine. Sodium tartrate, which stands at the opposite side of the series, was claimed to synergize morphine. Overcoagulation of brain colloids was made responsible for manic depressive insanity, overpeptization for stuporous catatonia.

We hope soon to communicate further researches dealing with the interrelation between colloidal changes produced by analgesics and antipyretics and their toxicologic behavior.

*As for quinine the pharmacologist, Rossbach[17] has observed more than half a century ago, that the hydrochlorate of quinine depresses the coagulation temperature of egg albumen, muscle extract and blood serum. But no attention was paid to this discovery; the less so, as the well-known biochemist Maly,[18] when reviewing this paper, emphasized, that the precipitation of proteins by the alkaloids does not mean anything else than the withdrawal of alkali, holding the proteins in a state of solution.

†As a water soluble substance closely related to pyramidon.

SUMMARY

1. A new functional liver test bearing an aromaturia (Fürth and Scholl), was applied to the study of phenylcinchoninic intoxication in animals. This test is based on the separation of certain phenolic urinary substances, yielding the Millon reaction. In one case of subacute phenylcinchoninic poisoning and in 6 cases of subacute and acute intoxication in rabbits with the ethyl-ester of p-methylphenylcinchoninic acid (Tolysin), there was no aromaturia, which if present would have indicated a severe injury of the liver. There only was aromaturia in a case of subacute phenylcinchoninic poisoning combined with hyperthyroidization.

2. In view of the alleged possibility of detoxication of phenylcinchoninic acid by a diet abundant in carbohydrates, some experiments were performed in albino rats: the minimal lethal dose in acute intoxication was nearly the same, whether the diet was rich in carbohydrates or entirely free from them. (But the negative outcome of these experiments does not prove anything for delayed intoxication in human beings.)

3. The toxicity of phenylcinchoninic acid for albino rats, guinea pigs, and rabbits was markedly increased in association with hyperthyroidism. The features of phenylcinchoninic intoxication are dominated by the injury of certain nervous centers, which perhaps primarily may be due to colloidal changes.

4. This view is supported by the fact that the diffusion of certain dyestuffs into gelatin is markedly increased by the presence of the phenylcinchoninic anion.

5. The swelling of gelatin in physiologic saline solution is markedly increased by the presence of phenylcinchoninic anion.

6. It seemed interesting to establish the position of the phenylcinchoninic anion in Hofmeister's anionic series, which are known to play an important part not only in colloidal chemistry, but also in many physiologic and pathologic phenomena. The phenylcinchoninic as well as the salicylic anion (R. Willheim) are to be placed beyond the end of the series

$$\left.\begin{matrix}\text{Phenylcinchoninic anion}\\ \text{Salicylic anion}\end{matrix}\right\} > CNS' > I' > NO_3' \quad \ldots\ldots$$

These observations may possibly contribute to understand the fact, that an abuse of phenylcinchoninic drugs can induce in predisposed individuals colloidal changes in the liver cells, perhaps beginning as slight cloudy swelling and (in some exceptional and unfortunate cases) ending in a yellow atrophy of the liver.

7. Besides the phenylcinchoninic and salicylic anion several substances, used as antipyretics, analgesics, and antirheumatics (quinine, antipyrine, phenocoll, melubrin) proved to accelerate the spontaneous coagulation of protein solutions, resulting from brain, muscle, and liver. It seems probable, that *some changes in the colloidal state (degree of swelling) of the proteins, building up certain nervous centers,* may play a part in the physiologic and pharmacologic behavior of these and related substances.

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A NOTE ON THE TREATMENT OF EXPERIMENTAL STREPTOCOCCUS MENINGITIS OF RABBITS WITH BACTERIOPHAGE*†

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THEORETICALLY at least the treatment of suppurative meningitis with bacteriophage would appear to offer a hopeful field for the clinical trial of this type of therapy, since it would appear possible to introduce sufficient amounts into the subarachanoid space to produce either lysis or the promotion of phagocytosis with special reference to the treatment of staphylococcus pneumococcus and streptococcus meningitis.

We are indebted to Dr. B. F. Stout of San Antonio, Texas, for suggesting this investigation on the possibilities of bacteriophage therapy in experimental meningitis and especially by subcutaneous and intravenous routes of administration, in view of his report on the recovery of a case of staphylococcus cavernous sinus thrombosis under this method of treatment.[1]

Fortunately our colleague, Miss Clara Kast, was able to produce a virulent bacteriophage for our strain of hemolytic streptococcus employed for several years in the production of a rapidly fatal type of meningitis in rabbits by intracisternal inoculation. So far, however, we have not been able to produce a virulent phage for our strain of Type I pneumococcus likewise employed for

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several years in the production of pneumococcus meningitis of monkeys, dogs, and rabbits. Our experiments, therefore, have been confined to the bacteriophage treatment of experimental streptococcus meningitis of rabbits.

In these animals the resulting infection is very rapidly fatal and extremely difficult to control. For example, the intracisternal injection of 0.5 c.c. of a 1:10 dilution of twenty-four-hour glucose brain hormone broth culture sometimes failed to produce meningitis altogether whereas the injection of a 1:5 dilution produced a severe purulent meningitis with septicemia ending fatally in eight to ten hours. As a general rule the rabbits were therefore inoculated with 1:6 to 1:8 dilutions and the resulting meningitis was usually fatal within twenty-four hours. In a few instances, however, untreated controls infected with these amounts of culture survived indefinitely, so that it was necessary to include a number of controls in each experiment and to exclude the results unless all untreated controls succumbed to the infection.

Treatment was started eight hours after intracisternal inoculation when the animals presented the severe classical signs of diffuse suppurative meningitis with purulent cerebrospinal fluids.

With some cisternal drainage was conducted about every twelve hours and the streptococcus bacteriophage injected intracisternally in dose of 0.5 to 0.75 c.c. per rabbit (approximately 0.2 to 0.4 c.c. per kilogram of weight). None survived and death usually occurred in forty-eight hours after treatment was instituted as shown in the results of an experiment summarized in Table I.

TABLE I

THE TREATMENT OF EXPERIMENTAL STREPTOCOCCUS MENINGITIS OF RABBITS WITH BACTERIOPHAGE

NUMBER OF RABBITS TREATED	PLAN OF TREATMENT	NUMBER OF TREATMENTS	SURVIVED	DIED	PERCENTAGE OF SURVIVALS
8	Cisternal drainage Bacteriophage intracisternally 0.5 to 0.75 c.c. Bacteriophage intracarotidly 2.0 c.c.	2	3	5	37
6	Cisternal drainage Bacteriophage intracisternally 0.5 to 0.75 c.c.	3	0	6	0
6	Cisternal drainage Bacteriophage intracarotidly 2.0 c.c.	2	2	4	33
16	None; controls	0	0	16	0

With others cisternal drainage only was conducted every twelve hours along with the injection of 1.0 c.c. of bacteriophage into each common carotid artery corresponding to the very large dose of about 1.0 c.c. per kilogram. With about 30 per cent of animals recovery followed the second or third injection and indicating that intracarotid injections were much more effective than intracisternal injections (see Table I).

Others still were given a cisternal drainage every twelve hours along with an intracisternal injection of bacteriophage in dose of 0.2 to 0.4 c.c. per kilogram and an injection of approximately 1.0 c.c. into each common carotid artery per kilogram. From 30 to 40 per cent of animals survived indefinitely (see Table I), so that this method of treatment gave the best results.

The results, therefore, are not without encouragement and particularly cisternal drainage combined with intracisternal and intracarotid injections of bacteriophage every twelve hours. So far the intracarotid injections have been most effective; unfortunately subcutaneous and intravenous injections have not yielded similar results in the few experiments conducted up to the present time. Smaller doses have likewise failed to yield similar results but this is largely a matter of concentration of virulent bacteriophage in the filtrates.

The time required and difficulties experienced in the preparation of autogenous streptococcus bacteriophage for the treatment of meningitis of human beings may militate against the application of this type of treatment in streptococcus meningitis of otitic or other origin but the results, so far, indicate the possibilities and merit serious consideration in the management of diffuse septic streptococcus meningitis in view of the extremely high mortality of this infection. As previously stated, we have not so far succeeded in producing sufficiently virulent bacteriophage for the different serologic types of pneumococci and no experiments have as yet been made with meningococci. The comparative ease of preparation of bacteriophage for staphylococci, however, greatly encourages the clinical trial of this type of therapy in staphylococcus meningitis.

SUMMARY

1. Cisternal drainage with intracisternal injections of bacteriophage have been ineffective in the treatment of severe, rapidly fatal diffuse streptococcus meningitis with septicemia of rabbits.

2. Cisternal drainage along with intracarotid injections of bacteriophage have resulted in the recovery of about 30 per cent of animals.

3. Cisternal drainage with intracisternal and intracarotid injections of streptococcus bacteriophage have resulted in the recovery of 30 to 40 per cent of animals, and it would appear that this method of treatment is worthy of trial in the treatment of diffuse streptococcus meningitis of human beings.

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SPONTANEOUS CARDIAC RUPTURE IN THE INSANE*

A Report of Six Cases†

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SIX cases of spontaneous rupture of the heart, occurring among 3257 deaths and 840 autopsies in a hospital for the insane, present an unusually high incidence of this dramatic fatality. Opportunity was taken for observations on associated changes, of which there is a dearth in much of the literature on this subject.

REPORT OF CASES

Case 1.—J. L., a white man, aged sixty-four, had been an inmate of the hospital ten years because of alcoholic dementia, hypertension, and peripheral vascular sclerosis. There was muscular weakness with tremor and numbness of the right side of the face and the right arm and leg; enlargement of the heart with muffling of the sounds. The urine had a fixed low specific gravity and contained albumin. On November 22, 1927, while performing his usual light tasks in the hospital bakery, he walked across the room, reached upward for his coat and fell forward dead.

Postmortem Examination.—The pericardial sac was completely distended with partially clotted blood. The heart (Fig. 1), weighing 590 gm., was elongated, firmly contracted, and infiltrated with subepicardial fat. On the anterior surface of the left ventricle, a linear ragged tear measuring 5 by 20 mm. roughly paralleled the long axis. It lay 30 mm. lateral to the anterior longitudinal sulcus and 35 mm. above the tip of the apex. In its center a small round opening admitted a probe directly and without resistance into the left ventricle. The inner aspect of the rupture appeared to the left and just below the origin of the anterior papillary muscle and the endocardium about it was covered with thin red thrombi. The cut surface of the myocardium was pale and streaked with fine white markings. The left ventricular wall was uniform in thickness and measured 18 mm.; the right, 8 mm. The valve rings were dilated. The chordae tendinae and tips of the papillary muscles were firm, thickened, and gray. The coronary arteries were rigid and atheromatous, and their lumina were irregularly diminished. The lower half of the anterior descending branch and the circumflex, as it turned right, was completely occluded by red thrombi. Other findings were chronic nephritis (together the kidneys weighed 100 gm.) and complete degeneration of the right cerebellar hemisphere.

Histologic Examination.—The coronary arteriosclerosis was marked and the thrombosis was recent. A moderate perivascular fibrosis was observed in all the myocardial sections. There was extensive anemic infarction of the myocardium of the left ventricle involving the entire wall, at the site of the rupture and in the adjacent portion those muscle fibers lying next to the endocardium. There was no myocardial inflammatory exudate, no attempt at organization nor any pericarditis. The endocardium about the rupture was covered with fresh thrombi.

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†The cases used as the basis of this report, including records of deaths and of autopsies, are from the Warren State Hospital.

CASE 2.—R. S., a white man, aged seventy-five, was in the hospital the third time because of a paranoiac condition and arteriosclerosis. One morning (Feb. 23, 1931) he was seized with excruciating abdominal pain followed by nausea and vomiting. During the afternoon the pain lessened somewhat. That night vomiting became more frequent and at 11:00 P.M., he suddenly died.

Postmortem Examination.—The pericardial sac was distended to its utmost capacity with partially clotted blood. The heart (Fig. 2) weighed 410 gm., was elongated, flabby, and infiltrated with epicardial fat. A jagged tear 2 by 30 mm. began at the tip of the apex and curved upward over the anterior surface of the left ventricle near to and roughly parallel with the anterior longitudinal sulcus. In its center was a small opening which admitted a probe directly and without resistance into the left ventricle. Surrounding the tear was a globular aneurysmal dilatation measuring 3 by 40 by 70 mm. which was studded with small hematomas. Viewed from within, the columnae carnae were stretched and flattened and the endocardium about the rupture was covered with thin red thrombi. There was infarction of the wall of the aneurysmal dilatation, of the adjacent posterior portion of the left ventricle and of the interventricular septum. The myocardium away from the rupture was mottled with coarse red and fine white streaks. The aortic ring was rigid and the mitral leaflets were covered with small, firm, translucent nodules. The distal two-thirds of the anterior descending branch of the left coronary artery, and the terminal branches of the circumflex were completely occluded by red thrombi. There was marked arteriosclerosis of both coronaries which began near their origins. Measurements: left ventricular wall away from rupture 20 mm., right, 5 mm. The valves were all dilated. Generalized arteriosclerosis, anthracosis of the lungs, and passive congestion of the viscera were present.

Microscopic Examination.—There was recent thrombosis of the blood vessels, fresh infarction of the wall of the cardiac aneurysm and adjacent muscle with cardiac endocardial thrombosis. Throughout the remainder of the myocardium there were interstitial and perivascular fibrosis and coronary arteriosclerosis. The pericardium was uninvolved.

CASE 3.—E. G., a white woman, aged sixty-six, was admitted to the hospital Feb. 14, 1931, because of a psychosis, senility, and neurosyphilis. The Kahn reaction on the blood serum was positive on two occasions. The heart was enlarged, the rhythm was irregular, and the blood pressure was 134/82. She became bedfast because of recurrent cardiac pain which radiated to the shoulders and was associated with circulatory collapse. She died suddenly in one of these attacks two weeks later (May 17, 1931).

Postmortem Examination.—The pericardial sac was greatly distended with partially clotted blood. The heart (Fig. 3) was short, wide, moderately infiltrated with fat and weighed 440 gm. A mottled infarcted, globular aneurysmal dilatation measuring 7 by 40 by 55 mm. occupied the midportion of the posterolateral border of the left ventricle. In its center was a smooth, small, round hole, which exuded blood on pressure. With difficulty a small probe was passed obliquely downward into the left ventricle where it appeared between the origins of the papillary muscles. Covering the endocardium about its inner aspect was a thin, easily detached thrombus. There was coarse brownish red and fine white streaking of the myocardium. A few firm translucent nodules occupied the free margins of the mitral leaflets. Measurements: wall of left ventricle away from rupture 30 mm., right ventricle 7 mm. The valves were dilated. The chordae tendinae and tips of the papillary muscles were thickened, gray, and rigid. The openings of the coronary arteries were partially obstructed by hyalinized aortic plaeques and puckered scars. The circumflex branch which ran directly into the infarcted area, the distal half of the left descending, and the right coronary artery just proximal to its bifurcation, were completely occluded by red and mottled thrombi, some of which could not be dislodged. There was marked generalized arteriosclerosis.

Microscopic Examination.—The aorta showed mesaortitis. In places the perivascular infiltrations of round cells were dense and sufficiently necrotic to suggest miliary gummas. There were extensive areas of hyalinization and of calcification which in places occupied

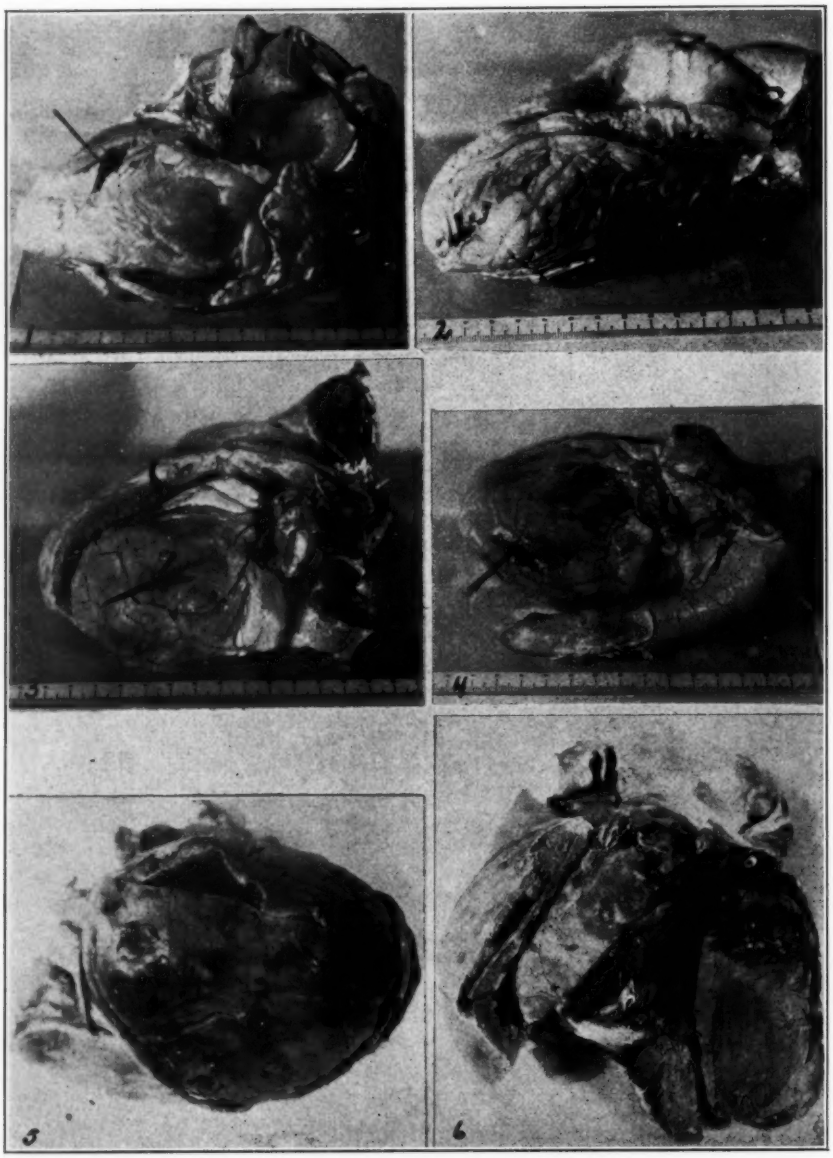

Fig. 1.—Heart Case 1. A probe has been inserted into the rupture, which has been sectioned.

Fig. 2.—Heart Case 2. A section has been cut from the linear tear which lies near the apex. Note the aneurysmal dilatation about the rupture and the subepicardial hematomas.

Fig. 3.—Heart Case 3. A probe has been inserted into the rupture which has been sectioned. Note the aneurysmal dilatation and the hematoma.

Fig. 4.—Heart Case 4. A probe has been inserted into the rupture. Note the frayed appearance about the rupture and the aneurysmal dilatation which surrounds it.

Fig. 5.—Heart Case 5. A probe has been inserted into the rupture. Note the extensive subendocardial hemorrhage.

Fig. 6.—Heart Case 5. A dissection showing the course of the tear and above it the large subendocardial hemorrhage. On account of the tortuosity of the rupture the section was made partially tangential to the muscle fibers.

four-fifths of the media. Sections from the wall of the cardiac aneurysmal dilatation consisted of partially organized necrotic muscle fibers, red blood cells, granulocytes, polyblasts, and pigment. There were thin endocardial thrombi and chronic pericarditis. The coronary arteries were arteriosclerotic and some were occluded by thrombi recent and old. The smaller branches were infiltrated with lymphocytes and showed subintimal fibrosis. A section through the line of rupture consisted of free and hemolyzed blood which extended into the crevices between the frayed and necrotic muscle fibers. Small infarcts in various stages of repair were scattered elsewhere in the myocardium.

CASE 4.—M. K., a white man, aged fifty-four, had been an inmate of the hospital half his life because of dementia precox. Physically he was strong and vigorous and regularly did hard outdoor labor. He appeared to be in good health although a blood pressure reading of 178/106 was recorded. At 3:30 A.M., Dec. 19, 1931, he complained of thoracic pain which, because of language difficulties, was not clearly evaluated. Three hours later his face was unusually ruddy and his gait was slow and labored. At 8:00 A.M. he had a severe generalized convulsion accompanied by intense cyanosis. He died twenty minutes later without regaining consciousness.

Postmortem Examination.—The pericardial cavity contained 500 c.c. of partially clotted blood. The heart (Fig. 4) weighed 350 gm. and was moderately infiltrated with fat. A linear frayed and ragged tear measuring 10 by 30 mm. occupied the lateral border of the left ventricle. It paralleled the long axis and admitted a probe without resistance directly into the chamber of the left ventricle. The heart wall surrounding the tear bulged to form a mottled brownish red aneurysmal dilatation which measured 3 by 45 by 75 mm. The pericardium about this was covered with thin easily detached friable material with numerous hematomas beneath. The inner aspect of the rupture appeared just to the left of the base of the anterior papillary muscle and was surrounded by thin thrombi. The muscle of the apex was completely replaced by tough fibrous tissue which was found in lesser amounts throughout the myocardium. Both coronary arteries were atheromatous and the opening of the left was partially obstructed by an aortic placque. Its branches were much thickened and their lumina diminished at many points. The circumflex branch which ran into the infarcted area was completely thrombosed 20 mm. from its origin. The left ventricular wall away from the dilatation measured 25 mm., the right, 7 mm. The mitral leaflets and base of the aortic cusps were thickened and rigid, and all the valves were dilated. There was generalized arteriosclerosis.

Microscopic Examination.—The changes in the aorta were characteristic of a mild mesaortitis and a marked degenerative arteriosclerosis. The coronary arteries were arteriosclerotic, and many were completely occluded by thrombi. In a section through the aneurysmal dilatation, the pericardium showed chronic inflammation, the endocardium was covered with thrombi and the myocardium was infiltrated with polyblasts, granulocytes, lymphocytes, and fibrin in all stages of necrosis. Away from the infarct, interstitial myocardial fibrosis was extensive. No evidence of syphilis was found in other organs.

CASE 5.—J. S., a white woodsman, aged seventy-six, was referred from the county home, March 15, 1926, because of senility, hypomania and chronic alcoholism of long duration. There was peripheral vascular sclerosis, hypertension and muffling of the heart sounds. Urinalysis, blood chemistry, and the blood Wassermann and Kahn reactions disclosed nothing abnormal. He contracted a febrile upper respiratory tract infection, and two days later was seized with excruciating thoracic pain which radiated to the arm and which subsided sharply within an hour. Almost immediately it was followed by deep cyanosis of the left side of the face and chest, inaudible heart sounds, impalpable pulse, and unconsciousness. In a few minutes he was dead, Feb. 24, 1932. A diagnosis of coronary occlusion and infarction of the heart with probable rupture was made.

Postmortem Examination.—The pericardial sac contained 500 c.c. of partially clotted blood. The heart (Fig. 5) was short and wide, moderately infiltrated with fat and weighed 560 gm. The visceral and parietal layers of the pericardium presented a buttered appearance. Both coronary arteries were tortuous, rigid, and atheromatous, and the right was completely

thrombosed 20 mm. from its origin. The entire posterior ventricular aspect of the heart presented a tarry mottling. An extensive subepicardial hematoma occupied the left auricular ventricular grove. Five millimeters to the left of the midportion of the posterior longitudinal sulcus was a smooth, round opening 2 mm. in diameter. After fixation a probe inserted into this extended upward and inward 18 mm., and then turned sharply into the chamber of the left ventricle. Here it appeared just inferior to the right border of the mitral orifice, as a slit-like fracture in a columna carnea attached to the wall along one side. Below and to the left of it the interventricular septum was infarcted and bulged into the right ventricle to form a small aneurysmal dilatation. The endocardium about it was covered with thin thrombi. On section the myocardium immediately surrounding the rupture was soft and mottled brownish red (Fig. 6); away from it there was fine white streaking. The interventricular septum and the line of insertion of the tricuspid valve was studded with subendocardial hematomas. The foramen ovale was patent, admitted a probe 4 mm. in diameter, but was well guarded by a dense fibrous valve. The free margins of the mitral leaflets were thickened and nodular. All the valves were dilated. The wall of the right ventricle measured 7 mm., of the left ventricle at the site of rupture 8 mm., and away from the rupture 15 mm.

Microscopic Examination.—The pericardium was covered with a fibrinous exudate which involved the areolar tissue about the base of the aorta. There was marked coronary arteriosclerosis and the right was completely occluded by a partially organized thrombus. The myocardium about the rupture was converted into a partially organized infarct. Chronic myocarditis with perivascular fibrosis was present in other parts.

CASE 6.—E. S., a white woman, aged sixty-five years, was admitted to the hospital July 16, 1895, with chronic mania. Some years before death she had been edematous, partially paralyzed, and suffered a fracture of the femur. Dec. 13, 1910, she was found dead in bed.

Postmortem Examination.—The incomplete protocol recorded that the pericardial sac was filled with partially clotted blood. The heart was hypertrophied and dilated. There was a slit in the myocardium 25 mm. in length which communicated with the right ventricle. Thrombi were found about the inner orifice of the rupture and also on the free margins of the tricuspid leaflets. About the base of the latter were calcific deposits.

COMMENT

Of these cases four were males and two were females. Their ages ranged between fifty-four and seventy-six years, the average being sixty-seven. The first five were encountered by one of us (Eaton) in a series of 140 routine autopsies performed at the Warren State Hospital between July 1, 1927 and Feb. 23, 1932, during which period there were 691 deaths. In the records of 2566 deaths and approximately 700 autopsies between Jan. 1, 1910 and July 1, 1927, only one such case was found. Since the files of the protocols are not entirely complete, other cases may have occurred. Combining these figures, there were six cases of spontaneous rupture in 840 autopsies and 3257 deaths. This is in keeping with the experience of Beresford and Earl of 31 cases in 2374 autopsies (Tooting Beck Hospital for mental and senile patients) and is sharply contrasted with that of others: Krumbhaar and Crowell 7 cases in 16,000 autopsies (Philadelphia General Hospital); Buckley 3 cases in 1330 autopsies (New Haven Hospital); and C. E. de la Chapelle 20 cases in 15,059 autopsies (Bellevue Hospital and Medical Examiner's Records, New York City).

Syphilis as a possible etiologic factor was noted in two cases. In Case 3 the evidence was indisputable. In Case 4 the partial obliteration about the origins of the coronary arteries and the histologic changes in the aorta were suggestive

although similar pictures have been described for other diseases (Pappenheimer and von Glahn, Moon and Stewart). The incidence of syphilis in spontaneous rupture varies from 4.5 per cent (Levine and Brown) to 50 per cent (Benson and Hunter). The latter group consisted of 14 ruptures out of 200 cases of fatal obstructive coronary artery disease, 17.5 per cent of which were syphilitic. In certain cases of rupture where the patients are known to have had the disease, it appears to play a minor rôle (Stewart), and when not combined with arteriosclerosis, the cases fall into a younger age group (Krumbhaar and Crowell). The occupations of our cases seem not to have had any bearing although 1 and 5 were alcoholics.

Cases 1, 4, and 5 had arterial hypertension, 2 and 3 did not. Peripheral vascular thickening was marked in 1, 3 and 5, but was not looked for in the others. All the patients were inmates of an institution for the insane. The patient in Case 4 did hard labor, and the patient in Case 1, although the victim of a prior paralysis, did light work up to the day of death. The others were decrepit and feeble. In Case 1 death occurred after the patient had walked a short distance and reached upward. The remaining five patients died in bed; two in an attack of acute cardiac insufficiency, one with and the other immediately following angina; one shortly after a convulsion which was preceded by angina; one after vomiting attacks which followed abdominal pain; and one during sleep. In Cases 1 and 6 death was sudden without premonitory symptoms; Cases 2 and 4 were known to be "cardiac cases." In Cases 2, 3, and 4 coronary occlusion was suspected and in 5 positively diagnosed. In our cases as in others (Longcope, Levine and Brown, Nathanson) and especially in the insane (Beresford and Earl), the clinical picture of coronary occlusion was not typical. No symptoms pathognomonic of rupture were noted, although it is interesting that in 5, after the experience with four previous ones, the diagnosis of probable rupture was made. An important sign of cardiac rupture is a sudden increase in the transverse area of cardiac dullness. In all our cases the pericardial sac contained above 500 c.c. of fluid.

It was not possible in any case to determine precisely the length of time which elapsed between rupture and death. In traumatic ruptures where the coronaries and myocardium are not diseased, there is a definite interval. In spontaneous cases, on the contrary, the diminished cardiac reserve is probably rapidly overcome by the added embarrassment to the already impaired circulation so that the interval, if one exists at all, must be short. Krumbhaar and Crowell have presented histologic evidence to show that this is not always so. Chapelle has reviewed the cases in which clinical evidence was presented to prove a definite interval: Engelhart one case, twelve hours; Curtin one case, twenty-four hours; Osler one case, thirteen hours; Quain eight cases, one eight days, one six days, one three days, and five of them over forty-eight hours. Chapelle believes it is probable that these were still in the stage of infarction. Contributing to the cardiac failure is tamponade of the heart due to the rapid rise in the intrapericardial pressure, disturbances in cardiac rhythm, cerebral anemia, peripheral vascular arteriosclerosis and shock resulting from hemorrhage.

The length of time elapsing between infarction and rupture is not so difficult to decide. Levine and Brown point out that the necrotic myocardium is softest between the fifth and fourteenth days. In three of our cases the infarct showed organization. In three others, judging from the histories and the postmortem appearances, rupture followed shortly after infarction.

In two cases the arteries occluded were the anterior descending and circumflex branches of the left coronary; in one case the circumflex branch alone; in another the right coronary and its branches; and in another (Case 3) all of them. In two cases the coronaries were partially occluded at their origins by changes in the aorta. Cardiac aneurysm was noted in four cases, and myocardial fibrosis in five. Pericarditis and partial organization of the infarcts and thrombi were noted in 3 cases. In one case coronary thrombi of various ages were found which confirmed the clinical impression of repeated occlusions.

The rupture: In five cases this occurred through the left ventricular wall and in one through the right. Case 5 is remarkable in that the rupture did not take place into the right ventricular chamber, for its general course was in that direction and only an abrupt change near the end brought it into the left. Of the first five, the tear was on the anterior surface near the apex in two; on the midportion of the lateral left border in two and near the midportion of the posterior longitudinal sulcus in one. These correspond to the common sites for cardiac infarct which Barnes and Ball have named, respectively, the anterior apical, the midventricular, and the posterior basal. In four the tear was linear, tortuous, or curved, and in two it consisted of a small round hole. In one this was partially covered by a thin flap. In all five a probe was admitted into the left ventricle. In two this passage was accomplished only after fixation and with some slight difficulty. The point is important for some ruptures are incomplete. In four the epicardial opening was larger than the endocardial while in one the reverse of this was true. In two the inner aspect of the rupture lay partially beneath the base, and in two others between the origins of the papillary muscles, while in one it appeared in a split of a columna carnea. The mechanism which actually brings about the rupture is usually thought to be a direct tearing of the muscle from the opposing pull of its own contraction or a giving way of the wall from pressure exerted upon it from within the chamber. The force of the former is much greater, although the latter is the factor in certain cases as one which followed malignant endocarditis complicated by aortic stenosis and a high intraventricular pressure (Tzankoff) which was somewhat similar to our last case. Important contributing factors are: the loss of support ordinarily rendered by the excessive epicardial fat before it undergoes necrosis, thinning of the wall by an aneurysm, ulceration of the endocardial surface of the infarct, and separation of muscle fibers by capillary hemorrhage with the formation of multiple hematomas. Beresford and Earl consider that the blood caught among the muscle fibers is forced onward by contractions, until a sinus tract is formed through the muscle and into the chamber. It seems of further importance to emphasize that in all our cases the mural thrombi, overlying the infarcted areas, were small and suggestively insufficient. In a previous case observed by one of us (Stewart), the endocardial surface of an extensive myocardial infarct was covered over by a thick thrombus except at the immediate

site of the inner aspect of the rupture, which remained unprotected. The connection between fatty infiltration and spontaneous rupture has been stressed. Earlier pathologists considered this as the direct cause, while recent writers tend to disregard it entirely. Beresford and Earl believe that an important relationship exists, since disturbance in lipoid metabolism is regarded as of etiologic importance in the senile psychoses. Davenport reports a high incidence of insanity in 92 recorded cases of spontaneous rupture. It is somewhat paradoxical that, in those of our series in which rupture occurred through the left ventricular wall, the subepicardial fat was largely confined to the right ventricle.

CONCLUSIONS

1. Six cases of spontaneous rupture of the heart occurring in patients who were inmates of an institution for the insane, are presented and discussed.

2. In five coronary arteriosclerosis, coronary thrombosis and myocardial infarction preceded the rupture, and in at least one of these syphilis was an etiologic factor.

3. In the insane the picture of coronary occlusion frequently is not typical.

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V. A DERMATOLOGIC TEST SUGGESTING AUTONOMIC IMBALANCE*

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SINCE tissue extract contains "desympatone,"[1, 2, 3, 4] a hormone capable of neutralizing epinephrine, we thought that, like epinephrine, it might be utilized as a test for autonomic imbalance. However, we have not seen any comparable systemic response upon administering "desympatone" in therapeutic doses. We then directed observation to local reactions caused by this complex mixture as well as various fractions of this product.

We found that tissue extract, among many fractions, contains one, apparently a polypeptide, which produces a very interesting cutaneous reaction in many individuals. Because of its constricting local action, we have called this fraction "S" or "strictor" substance. It is not histamine, choline, or "desympatone." It is thermostable, is inactivated by alkalies and reactivated by acids.

It is prepared in the following manner:

Pancreas, kidney, or any other tissue is extracted with a hydroalcoholic menstruum containing hydrochloric acid. The extract is neutralized with ammonium hydroxide and filtered. The filtrate is acidified and concentrated in vacuuo. Ammonium sulphate is added to the clear concentrate yielding a precipitate which contains the "S" constituent. This precipitate is extracted with alcohol, and any insulin removed if pancreas is used. The solution is concentrated in vacuuo and passed through a Berkefeld filter leaving a residue which contains most of the "S" substance. This residue is dissolved in an acid menstruum to give a 3 per cent solution.

Different people and animals react differently to subepidermal injections of graded amounts of this substance.

The test is performed as follows: Of the solution, 0.025 c.c. is injected subepidermally (not intradermally) by means of a 26 gauge needle (Schick type) and a tuberculin syringe (Fig. 1). A bleb is produced (Fig. 2) (at times pseudopodia develop), and in the center a pearly white area appears (Fig. 3). Histologically we have found this to be due to focal tissue changes.† This usually develops in three to five minutes after injection, and is considered a "positive" reaction, providing it remains for more than twenty-four hours (Fig. 4). A "negative" reaction is one which fails to show this characteristic white center if 0.025 c.c. is injected (Fig. 5) or disappears in less than twenty-four hours. We

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have arbitrarily adopted this as our test dose. If a sufficiently large quantity is injected, a "positive" reaction may be obtained in most instances.

The more "S" substance required to produce this reaction, the more "negative" we consider the individual (Fig. 6).

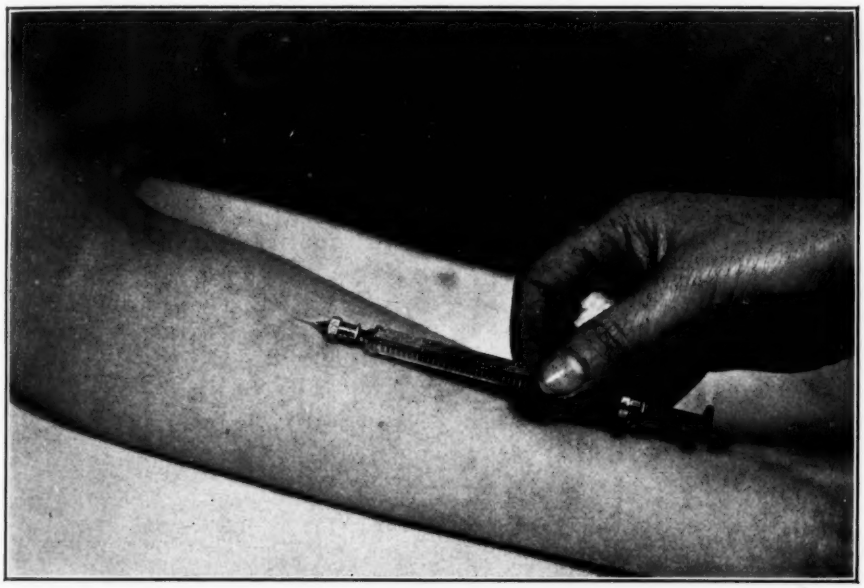

Fig. 1.—Twenty-six gauge needle (Schick type) using a tuberculin syringe.

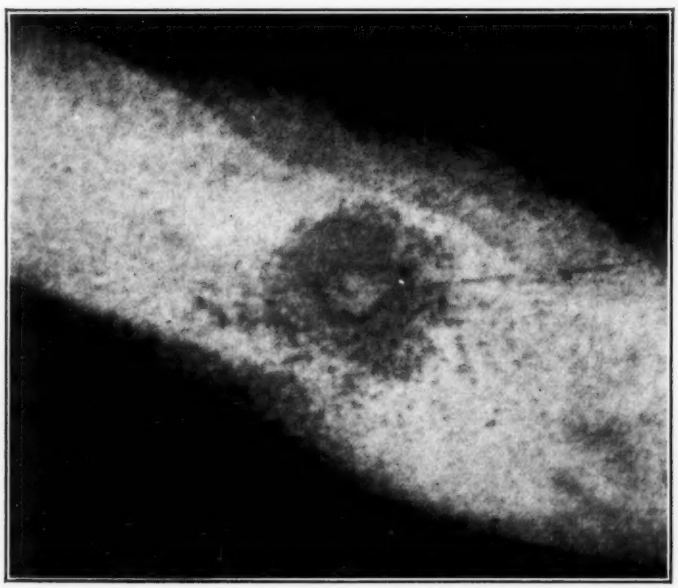

Fig. 2.—A bleb is produced with a central pearly white area just developing.

If 0.025 c.c. fails to yield a "positive" reaction, the dose is gradually increased to 0.05 c.c., 0.075 c.c., 0.1 c.c., etc., until a "positive" reaction is obtained.

After testing over 500 normal individuals and hospital patients, we were impressed with the fact that apparently the so-called "vagotonic," or parasympatheticotonic individuals react characteristically to a small amount of "S" substance such as 0.025 c.c. or even less.

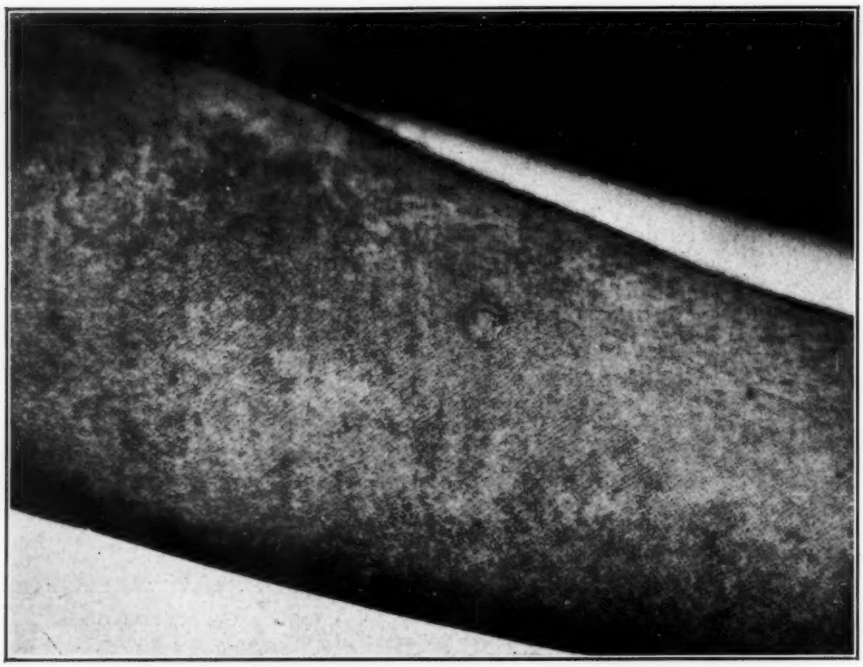

Fig. 3.—Central area in a further state of development.

Fig. 4.—Twenty-four hours after injecting "S" solution. Response is shown to ascending amounts of the substance, viz.: *I*, 0.001 c.c.; *II*, 0.0005 c.c.; *III*, 0.01 c.c.; *IV*, 0.025 c.c.

So far, we have been unable to duplicate this reaction with substances such as milk, sera, histamine and many others. The ease with which this test may be carried out and read may encourage others, particularly pharmacologists, to study it. More detailed findings will be published later.

Fig. 5.—*I*, immediately after injection. *II*, Five minutes after injection.

Fig. 6.—This patient had jaundice, and therefore the pseudopodia show up more definately. *A*, Shows a very minute response to 0.075 c.c. *B*, Shows a marked response to 0.2 c.c.; eight times as much substance as it would take a positive case to give a similar reaction.

CONCLUSION

An "S" or "strictor" substance extracted from various tissues brings about different reactions in different individuals and animals. By a standard technic herein reported, apparently some individuals have given a "positive"

reaction, while others have given "negative" reactions. While suggestive that the reaction is influenced by the vegetative state, there is nothing conclusive to warrant its general adoption as a test until further researches are made.

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AN EVALUATION OF THE EFFICACY OF OLEIC ACID WITH BILE SALTS IN ENTEROHEPATIC DISEASE*

A Clinical Experiment with Twenty-five Subjects

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THE function and mechanism of the gall bladder and the manner of the emptying of bile into the duodenum are still the subject of much bewildering conjecture. It is an elusive and fascinating jungle trail which is being trekked persistently by a score of investigators in as many experimental laboratories.

Since the intimate relationship of hepatic to alimentary function has become established, the result of these investigations is increasingly significant because of its therapeutic import. Age or other physical disability forbids the benefit of surgical relief to one group of biliary tract sufferers. There are a considerable number of patients who for economic or other reasons will forego any medical assistance rather than agree to hospitalization or to bed management. Even with those who do consent to surgery, there is the well-known disturbing percentage of postoperative symptomatic recurrence.

The problem of the medical management of these cases becomes an imperative issue when one includes the large number of patients whose only symptoms are dyspeptic, for which no corresponding organic lesion can be demonstrated. Blackford and Dwyer[1] have found from their study of 1650 cases of chronic dyspepsia, that gall bladder disease occupies first place as a single cause of stomach complaint. They estimated that all organic stomach diseases together caused only 14.3 per cent of gastric symptoms, as against 20.4 per cent caused by derangement of the gall bladder. In their series, the approximate relative frequency of abdominal organic lesion causing dyspepsia was gastric ulcer, 1; gastric carcinoma, 2; reflex appendicitis, 4; duodenal

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ulcer, 6; *gall bladder disease, 12.* Dowling[2] in a later study of 1004 cases of gall bladder or bile duct disease found that the only early and prominent symptoms were dyspepsia in 64.5 per cent of these patients. It is these early dyspeptic patients who need the benefit of a rational medical procedure. It is logical to believe that such a therapeutic course is prophylactic against more serious involvement as well as alleviative.

Hence, there is an evident need for a medical mode of treatment which can be "proved up" in accordance with our newer experimental knowledge of biliary tract mechanism.

With this purpose in view, Lipschutz[3] in 1927 presented an experimental study of the efficacy of oleic acid with bile salts in emptying the human gall bladder and of stimulating a depleted bile flow, so as to evaluate the utility of such a medical treatment for relief of the biliary and gall bladder stasis which is a common factor in these cases.

In the present instance I have made a study of twenty-five subjects, using a different experimental approach so as to confirm or deny the previous findings. My results are the subject of this paper.

THEORETICAL BASIS OF THE EXPERIMENT

While it is not within the scope of this paper to review the divergent opinions which have been raised experimentally with regard to gall bladder mechanism, it will be explanatory of the theoretical basis of my series of experiments to recall certain measures of progress which have been made by various investigators with typically constant results.

To begin with, two momentous contributions have made it possible to evaluate human reactions more readily, which hitherto were not observable except by empirical conclusion. The first of these was the introduction of diagnostic (and therapeutic) nonsurgical biliary drainage by Lyon[4] in 1919, by which it is possible through use of the duodenal sound to observe the rate of discharge of bile into the bowel and the character of the biliary fluid. Lyon[5] has recently demonstrated further (1929) that good specimens of gall bladder bile may be obtained by duodenal tube after intraduodenal stimulation with certain substances and that in patients with unobstructed cystic ducts and less advanced cholecystitis, the gall bladder is the source of B bile and empties itself after certain intraduodenal stimulation.

The second contribution was the introduction by Graham, Cole and Copher[6] in 1924 of a method of visualization by the introduction into the body of dyes opaque to x-rays, which because of their concentration in the gall bladder make cholecystography practicable.

Various experiments, animal and human, have been reported to indicate the effect of foodstuffs and drugs upon both the secretion of bile and its emptying into the bowel. Of the types of food which encourage emptying of the gall bladder, it has been uniformly observed that fats are apparently unique. For the convenience of the reader, reference is made in the bibliography to some of the investigators who have arrived experimentally at this conclusion.[7-11] More recently, Babkin and Webster[12] in 1929, Higgins and Wilhelmj[13] in 1929,

and Bassin and Whitaker[14] in 1930 have experimentally corroborated the earlier findings with regard to the dynamic influence of fats and fatty acids in promoting emptying of bile into the duodenum. Thus Bassin and Whitaker conclude that "fats in emulsion, given either by mouth or intravenously, appear to be the most constant and effective means of evacuating the gall bladder."

It would, then, be logical but not practicable to feed the patient with fat-rich meals, were they not so poorly tolerated. Administration of oleic acid is, however, as extraordinarily effective in respect to evacuation of bile, and in addition eliminates the unavoidable disadvantages accompanying forced fat feeding. The efficacy of fatty acid as a gall bladder evacuant has been demonstrated by Copher and Kodama (1926),[15] Ivy and Oldberg (1928),[16] Stewart and Ryan (1928),[17] Ivy (1929),[18] and Crandall (1931).[19] Thus Stewart and Ryan, making cholecystographic observations after administration of fourteen different drugs, found that the most constant response in the evacuation of bile from the gall bladder was obtained from oleic acid. They did not find that bile salts produced any evacuation.

Since there is usually a degree of diminution of bile secretion as well in the presence of enterohepatic stasis, therapeutically it appears rational to administer both oleic acid and bile salts whenever enterohepatic symptoms apparently due to biliary insufficiency indicate the advisability of medical treatment. Empirically and experimentally, bile salts have long been known as powerfully choleretic (promoting the secretion of bile, in contradistinction to cholagogic action or the evacuation of bile from the gall bladder). Recently, the experimental choleretic response after bile salts in dogs has been reported by Chabrol and Maximin[20] in 1929, and by Puestrow[21] in 1931. The former found that the secretion of bile tripled after the intravenous injection of bile salts; and the latter, that "bile salts administered intravenously produced a profuse flow of thin yellow bile which lasted more than three hours."

EXPERIMENTAL

In the group of experiments conducted by Lipschutz,[3] he administered oleic acid in conjunction with bile salts* to patients, and observed the efficacy of such a dosage in evacuating the gall bladder after administering the dye, sodium tetraiodophenolphthalein salt, and taking cholecystograms. The results were persistently uniform. Two hours after the ingestion of five capsules the cholecystograms invariably showed a diminution in the gall bladder shadow from one-half its original size to almost complete disappearance.

These experiments did not, however, afford a means of measuring any possible quantitative increase in bile secretion and the amount of bile evacuated into the duodenum. This I have endeavored to cover in my present experiment. In other words, I have tried to ascertain both the choleretic and the cholagogic influence of such a dosage.

I used in my experiment 25 patients (20 females and 5 males). Sixteen were sufferers from chronic cholecystitis. Three others had peptic ulcer; 4 spastic colitis; and there were 2 normal controls.

*Each soluble elastic capsule contained oleic acid 15 mins., sodium taurocholate ¼ gr., and sodium glycocholate ¼ gr.

The purpose of the experiment was to ascertain by biliary drainage in each case over a two-hour period the amount and kind of bile secured without stimulation after fasting; to ascertain on a second nonconsecutive day, the same data after stimulation with bile salts; and on a third nonconsecutive day, the same data after stimulation with oleic acid and bile salts in conjunction.

The technic on each day was as follows: After a fourteen-hour fast overnight, the subject was prepared for duodenal drainage with a Jutte duodenal tube and placed in the Trendelenburg position so as to facilitate collection of the duodenal contents without loss. I have found this tube preferable because of the ease of passage of its small metal tip through the pylorus and its more ready facility in evacuating the duodenal contents through multiple perforations. During manipulation, the position of the tube was observed fluoroscopically. When it was in position, the duodenum was washed out with 10 c.c. of warm water administered through the tube and the tube clamped off. After ten minutes, the tube was unclamped and drainage of the duodenal contents continued for two hours, without suction and with each hourly quantity in the drainage collection bottle measured separately.

On the second nonconsecutive day of the experiment, the observations were made after the administration through the tube of 3 gr. of bile salts in 10 c.c. of warm water.

On the third nonconsecutive day of the experiment, the observations were made after duodenal administration of 6 c.c. of oleic acid in conjunction with 3 gr. of bile salts.

OBSERVATIONS

Table I gives the individual results.

1. Of the 25 subjects, 20 showed a greater outpouring of bile into the duodenum after bile salts than without stimulation of any kind. Of the 25, 24 showed a greater outpouring of bile after oleic acid with bile salts than without stimulation of any kind. The bile evacuation was considerably greater after oleic acid with bile salts than after bile salts alone, there being but one case where the former result was less than the latter.

2. B bile was not obtained in any case without stimulation or in any case after bile salts. It was typically obtainable after oleic acid with bile salts, being absent in only three cases. This corroborates the proposition of the dynamic effect of oleic acid upon gall bladder evacuation, if we are to consider as conclusive Lyon's observations[5] that the gall bladder is the source of B bile. The average amount of B bile obtained was 24.32 c.c. It was typically obtainable within five minutes after the stimulative influence was applied.

3. These experiments tended to show also that oleic acid is choleretic as well as cholagogic, and that therefore it augments the secretory effect of bile salts upon the liver. One comes to this conclusion by comparing the quantity of bile (other than B or gall bladder bile) obtainable after oleic acid with bile salts, with the quantity of bile obtainable after bile salts alone. The amount of bile obtainable in the former case is typically a greater amount than in the latter case, there being but three exceptions in my series of 25 cases.

TABLE I

AMOUNT OF DUODENAL BILE PROCURED

CASE	WITH NO STIMULATION	AFTER BILE SALTS STIMULATION	AFTER OLEIC ACID AND BILE SALTS STIMULATION		
			AMT. BILE OTHER THAN B BILE	AMT. B BILE	TOTAL
1	35 c.c.	40 c.c.	75 c.c.	15 c.c.	90 c.c.
2	53 c.c.	68 c.c.	165 c.c.	0 c.c.	165 c.c.
3	17 c.c.	25 c.c.	40 c.c.	10 c.c.	50 c.c.
4	80 c.c.	92 c.c.	120 c.c.	22 c.c.	142 c.c.
5	92 c.c.	80 c.c.	75 c.c.	8 c.c.	83 c.c.
6	40 c.c.	55 c.c.	73 c.c.	12 c.c.	85 c.c.
7	95 c.c.	115 c.c.	240 c.c.	23 c.c.	263 c.c.
8	102 c.c.	118 c.c.	172 c.c.	15 c.c.	187 c.c.
9	112 c.c.	105 c.c.	100 c.c.	25 c.c.	125 c.c.
10	29 c.c.	25 c.c.	42 c.c.	10 c.c.	52 c.c.
11	0 c.c.	12 c.c.	33 c.c.	12 c.c.	45 c.c.
12	0 c.c.	31 c.c.	25 c.c.	0 c.c.	25 c.c.
13	123 c.c.	135 c.c.	285 c.c.	100 c.c.	385 c.c.
14	111 c.c.	128 c.c.	240 c.c.	75 c.c.	315 c.c.
15	87 c.c.	95 c.c.	137 c.c.	32 c.c.	169 c.c.
16	12 c.c.	0 c.c.	132 c.c.	0 c.c.	132 c.c.
17	180 c.c.	240 c.c.	315 c.c.	45 c.c.	360 c.c.
18	144 c.c.	152 c.c.	211 c.c.	35 c.c.	246 c.c.
19	300 c.c.	322 c.c.	387 c.c.	27 c.c.	414 c.c.
20	172 c.c.	210 c.c.	245 c.c.	32 c.c.	277 c.c.
21	93 c.c.	111 c.c.	178 c.c.	22 c.c.	200 c.c.
22	242 c.c.	264 c.c.	341 c.c.	17 c.c.	358 c.c.
23	42 c.c.	73 c.c.	192 c.c.	13 c.c.	205 c.c.
24	143 c.c.	128 c.c.	183 c.c.	21 c.c.	204 c.c.
25	278 c.c.	294 c.c.	350 c.c.	37 c.c.	387 c.c.
Total	2582 c.c.	2918 c.c.	4356 c.c.	608 c.c.	4964 c.c.
Average	103.28 c.c.	116.72 c.c.	174.24 c.c.	24.32 c.c.	198.56 c.c.
Average increase %		13%			92.2%

4. With regard to percentage of increase in amount of bile obtainable after stimulation, in the case of stimulation with bile salts, omitting 4 anomalous cases, the lowest increase was 5.5 per cent; the highest, 73.8 per cent; and the average increase of all 25 cases, 13 per cent. In the case of stimulation with oleic acid in conjunction with bile salts, omitting 4 anomalous cases, the lowest increase was 11.6 per cent; the highest, 388 per cent; and the average increase, 92.2 per cent.

5. Of the atypical reactions, the following were observed. In Case 5, both bile salts alone and oleic acid with bile salts failed to produce as much duodenal bile as was obtained without stimulation. In 4 other cases (9, 10, 16, and 24), bile salts alone failed to produce bile increase, although oleic acid with bile salts effected some increase. In one anomalous case (12) oleic acid with bile salts produced less bile increase than did bile salts alone, and produced no B bile. There were two other instances in which B bile was not procured after oleic acid with bile salts.

6. Table II presents percentages of increase, and Chart 1 compares graphically the average bile measurements procured in the three different phases of my experiment.

7. I realize that there are controversial views with regard to the accuracy of duodenal drainage as a method of recovering all of the bile from the duodenum. A point in favor of its accuracy is the uniformity of the results I secured in my 25 experimental cases. It is the opinion of some investigators[22] that this method of drainage procures from 94 to 99 per cent of duodenal increment.

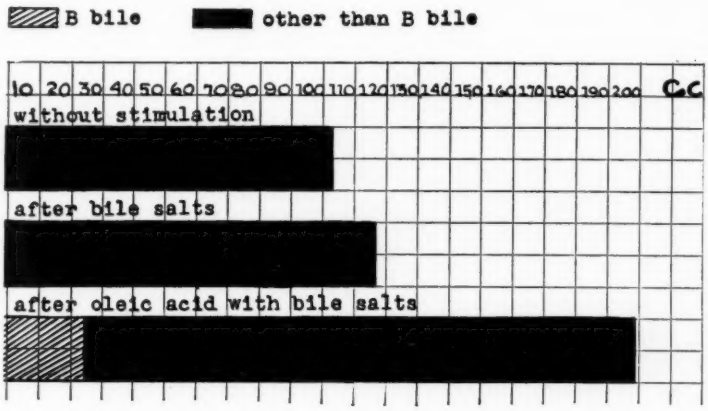
B bile
other than B bile
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 C.C
without stimulation
after bile salts
after oleic acid with bile salts

Chart 1.—Average cubic centimeters of bile recovered from duodenum in twenty-five subjects.

TABLE II

INCREASE OF BILE AFTER STIMULATION OVER AMOUNT SECURED WITHOUT STIMULATION

CASE	AFTER BILE SALTS	AFTER OLEIC ACID WITH BILE SALTS
1	14.3%	157.1%
2	28.3%	211.3%
3	47.0%	194.1%
4	15.0%	77.5%
5	----*	----†
6	37.5%	112.5%
7	21.0%	176.8%
8	15.6%	83.3%
9	----	11.6% low
10	----	79.3%
11	Approximately 1200%*	Approximately 4500%†
12	Approximately 3100%*	Approximately 2500%†
13	9.7%	213.0%
14	15.3%	183.7%
15	9.2%	94.2%
16	----*	1000.0%†
17	33.3%	100.0%
18	5.5% low	70.8%
19	7.3%	38.0%
20	22.0%	61.0%
21	19.3%	115.0%
22	9.0%	47.9%
23	73.8% high	388.0% high
24	----	42.6%
25	5.7%	39.2%

Average percentage of increase

After bile salts ---------- 13.0%
After oleic acid with bile salts ---------- 92.2%
(figured by averaging totals of c.c. in Table I.)

*Cases considered anomalous. Omitting these anomalous cases, the lowest percentage of increase was 5.5%; the highest, 73.8%.

†Cases considered anomalous. Omitting these anomalous cases, the lowest percentage of increase was 11.6%; the highest, 388%.

My experiment has corroborated the observations of Lipschutz[3] and Levinson,[23, 24] who also experimentally and clinically found that administration of oleic acid with bile salts is an effective means of relieving biliary stasis.

SUMMARY AND CONCLUSIONS

The bile procurable by duodenal drainage was measured as follows in 25 subjects: on the first day, without duodenal stimulation; on the second nonconsecutive day, after duodenal administration of bile salts; and on the third day, after similar stimulation with oleic acid and bile salts in conjunction.

There was a typical increase in bile flow after bile salts, and also a typical and much greater increase after oleic acid with bile salts. In the former instance, the average percentage of increase was 13 per cent; in the latter, 92.2 per cent.

B bile (gall bladder bile) was procured only after stimulation with oleic acid and bile salts in conjunction.

Barring the inaccuracies due to unknown factors, the experiment demonstrated that oleic acid is strongly dynamic as a cholagogue (evacuating the gall bladder) and also is definitely dynamic as a choleretic (increasing bile secretion); and that bile salts are dynamic as a choleretic and not at all as a cholagogue.

Therapeutically, it suggested that the experiment demonstrates the preferable efficacy of administering oleic acid and bile salts conjointly whenever medical treatment is in order in enterohepatic disease accompanied apparently by biliary stasis.

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57 WEST FIFTY-SEVENTH STREET.

CARCINOMA OF THE STOMACH WITH METASTASES TO THE SKIN*

CASE REPORT

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GENERALIZED metastases to the skin from carcinoma of the stomach is not a usual occurrence. A careful search of both the domestic and foreign literature reveals not more than 137 such cases. Nobuyoschi Suzuki[1] in commenting upon the rarity of this condition states that Reichelman reviewed a series of 711 cases of gastric cancer, and Gussenbauer and Winiwarter studied 903 such cases and failed to find a single case presenting secondary tumors to the skin. Lubarsch, quoted by Guilbert,[2] found only 0.4 per cent of a series of 2738 cases of gastric cancer metastasizing to the skin. When such metastases occur, they are found more commonly in the region of the umbilicus. In only a few of the reported cases were the nodules very numerous. Of all the abdominal cancers metastasizing to the skin, those of gastric origin, according to Bourquelot,[3] hold the first place, while intestinal and uterine cancers come next in frequency. Complete case reports have been presented notably by Crouzon,[4] Loeper and Turpin,[5] Menetrier and Gaukler,[6] Merklen,[7] Maunoury,[8] Durbeck,[9] Suzuki,[10] and Uhlenbruch and Gilardone.[11]

The following case report is presented including complete necropsy findings.

CASE REPORT

History.—The patient, a colored female, aged forty-two, was admitted to the medical service of the Montefiore Hospital on October 24, 1932, complaining of marked dyspnea and pain in the abdomen. She felt perfectly well until three months previous to her admission when she began to be troubled with a slow, dull, aching pain in the upper abdomen, this

*From the Medical Service of the Montefiore Hospital.
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pain radiating to the small of the back. It usually occurred about one hour after meals, and was relieved chiefly by vomiting. Her appetite was very poor. There was no history of hematemesis or blood in the stools. Her bowels moved only following catharsis. About the time of the onset of the above symptoms, she noticed a gradual but painless enlargement of her abdomen. One month previous to her admission she began to notice variable sized masses occurring in the skin over the anterior surface of the chest and abdomen. During this interval she lost about fifty pounds in weight. For a few months she had also been troubled with increasing shortness of breath on exertion. There was no edema of the ankles, no cough, and no hemoptysis. She presented no symptoms referable to the respiratory or urinary systems. Her menstrual periods were irregular. She had been married for thirteen years but was never pregnant. The past medical history included measles, mumps, pneumonia, typhoid fever, and influenza. There was no history of venereal diseases, repeated sore throats, skin rash, or miscarriages. Her father was living and well. Her mother died at forty-five due to childbirth. There was no history of tuberculosis or cancer in the family.

Physical Examination.—The patient was an adult, colored female, weighing about 110 pounds. Temperature 98°, pulse 120, respirations 22, blood pressure 140/80. On admission she appeared emaciated, pale, apparently acutely ill, and suffering from a great deal of pain. The skin over the entire chest and abdomen, anteriorly and posteriorly, was covered

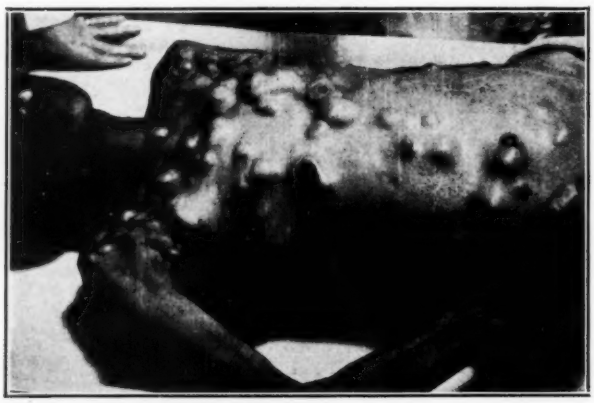

Fig. 1.—Anterior view showing metastasis to skin.

with numerous irregular, hard, subcutaneous nodules varying in size from a pea to a walnut (Fig. 1). Some of these nodules were freely movable beneath the skin, and others were more firmly attached. Over several of these the skin was atrophic and showed signs of chronic irritation with a tendency to ulceration. There was one walnut-sized tumor over the left malar process, and several over the right thigh. Supraclavicular nodes were not palpable. The pupils were unequal, the left being larger than the right, and the right was definitely irregular. Reaction to light was sluggish but both pupils reacted to accommodation. Posterior synechia were present. On the right side there was moderate retinal arteriosclerosis. Otherwise the fundi were negative. The ears and nose were negative. The mouth was dentitionless. The tonsils appeared imbedded and slightly cryptic. The submaxillary lymph nodes were enlarged and palpable. Heart and lungs were negative. The abdomen showed definite enlargement which was apparently due to a massive involvement of the liver so that the edge of the liver extended about 3 cm. below the umbilicus. The organ appeared hard, firm, and suggestively nodular on palpation. The left kidney region presented some tenderness. The spleen was not palpable. The knee jerks were equal but decreased. No pathologic reflexes were present. Pelvic examination revealed the presence of infantile pelvic organs and a retroverted and adherent uterus.

Laboratory Examination.—Urine was negative. Phenolsulphonephthalein, first specimen, 12.2 per cent; second specimen, 9.5 per cent; total 21.7 per cent. Blood chemistry: sugar,

93 mg., nonprotein nitrogen, 31 mg. Several blood counts performed during the patient's stay in the hospital showed a progressive anemia; thus, on Oct. 26, 1931: hemoglobin, 60 per cent; R.B.C., 3,400,000; W.B.C., 8000; differential, normal. On Nov. 18, 1931: hemoglobin, 25 per cent; R.B.C., 1,680,000; W.B.C., 8000; differential, normal. Wassermann and Kahn tests were negative. Following a provocative test the blood Wassermann became positive and remained positive throughout the patient's stay in the hospital. Stool examinations were negative for blood and parasites. Fractional gastric analysis was negative for

Fig. 2.—Section of skin nodule (low power), showing carcinomatous infiltration.

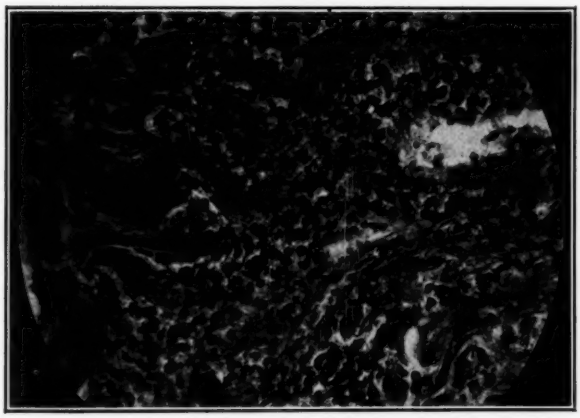

Fig. 3.—Section of skin nodule (high power).

lactic acid, but positive for blood; the specimen showed achlorhydria and a low combined and total acidity. Gastrointestinal roentgenographic study showed a constant filling defect at the pyloric end of the stomach. The character of this defect was suggestive of an annular carcinoma of the pars pylorica. X-ray study of the colon by aid of barium enema revealed a slight narrowing of the descending colon about five inches from the rectosigmoidal junction. There was some ptosis of the colon but no evidence of any permanent obstruction. A roentgenogram of the left maxillary bone taken from the region of the subcutaneous nodule failed to reveal any metastases to the bone. Biopsy was made of one of the skin nodules.

Grossly the specimen submitted consisted of two irregular masses covered with adipose tissue. On section they were firm and consisted of a pearly gray tissue infiltrating the surrounding structure. Microscopically the structure was composed of epithelial-like cells which infiltrated the stroma in all directions. The nuclei were hyperchromatic, and numerous mitotic figures were seen. The stroma was dense connective tissue. Scattered through the tumor were areas of degeneration and lymphocytic infiltration. Generally, the picture was that of carcinoma with degeneration (Figs. 2 and 3).

Diagnosis.—Carcinoma of the stomach with generalized metastases to the skin and liver. Syphilis. Secondary anemia.

Course.—The patient was given symptomatic treatment. In spite of the positive serology it was felt that syphilis did not play an important rôle in the existing clinical condition. Therefore, because of this consideration as well as because the patient was so acutely ill, antisyphilitic treatment was not forced. The course of her condition was uneventful until November 11, or about one month following her admission, when she suddenly developed a severe gastric hemorrhage, vomiting bright red blood. She recovered sufficiently from this hemorrhage and got along fairly well until January 3, when she complained of a sudden abdominal pain after which she became pulseless, showed all the signs and symptoms of severe shock, and then died in two hours.

Necropsy.—The body was that of an adult colored female showing marked emaciation. The skin of the chest wall, anteriorly and posteriorly was studded with firm round tumor masses, some of them fixed and others movable. The skin covering the tumor masses was intact, except in two places where ulceration was seen. The abdominal wall had similar nodules, but fewer in number. The extremities showed only two similar nodules, in the left thigh. There was a large lump the size of an orange in the left zygomatic region, fixed to the bone. The eyes, ears, and nose were grossly negative. The primary incision passed through a tumor mass on the chest wall. The cut surface of the tumor showed central necrosis. The peritoneal cavity contained about 2 liters of brownish-red slightly turbid fluid. The parietal peritoneum was studded with numerous nodules. The abdominal viscera were found in their normal position and relation, but adherent to each other, with old and new adhesions partly due to neoplastic involvement, and partly to recent peritonitis. Thorax: The lungs were semicollapsed. The right pleural cavity contained about 300 c.c. of serous fluid. The left cavity was free of fluid. There were numerous fine old adhesions between the layers of the pleura. The pericardial sac contained the usual quantity of straw-colored fluid. Both lungs were identical in gross pathology. They had the usual anthracosis of the district and several foci of healed lesions of tuberculosis and moderate degree of terminal edema. No macroscopic evidence of neoplastic involvement was found. The peribronchial lymph nodes showed slight enlargement and discoloration. One was involved with tumor growth. The heart was rather small, and the pericardial covering was smooth. The myocardium was pale and flabby. The endocardial lining was smooth. The aortic valve showed moderate thickening and atheromatous changes. The other valves were normal. The arch of the aorta was moderately dilated and the intima showed longitudinal striations. The opening of the coronary vessel was patent, and the vessels were free from atheromatous changes. Abdomen: The liver was markedly enlarged and distorted. It weighed 3.2 kg. The right lobe was extensively involved with tumor growth. The surface of the liver was rough and nodular. More than half of the liver parenchyma was replaced by tumor masses. These were pearly-gray, very firm in consistency, with a tendency to central necrosis. The left lobe of the liver was free from such involvement. The gall bladder and the large biliary ducts were normal. The ampula of Vater was patent. The pancreas was normal. The tail of the pancreas was adherent to the spleen and to the upper pole of the left kidney. The spleen was moderately hyperplastic. The capsule was thick and was infiltrated with several tumor nodules. The splenic tissue proper showed moderate degree of congestion and softening, but no involvement by tumor. The left kidney showed nodules at the upper pole. The rest of the organ was pale. No definite line of demarcation was evident between the cortex and the medulla. The right kidney was free from metastasis. The internal genitalia

were essentially negative except for a few old adhesions. The ovaries were atrophic, and no neoplastic changes were present. Gastrointestinal Tract: The stomach was collapsed. There was a perforation in the lesser curvature, about 4 cm. from the pylorus on its anterior aspect. On opening the stomach, there was a firm tumor mass the size of a silver half dollar. This mass showed ulceration in the center, with a perforation measuring 2 mm. in diameter. The lymph nodes both at the lesser and greater curvatures showed metastatic involvement. The mesenteric lymph nodes were generally involved. The lumina of the intestines were free

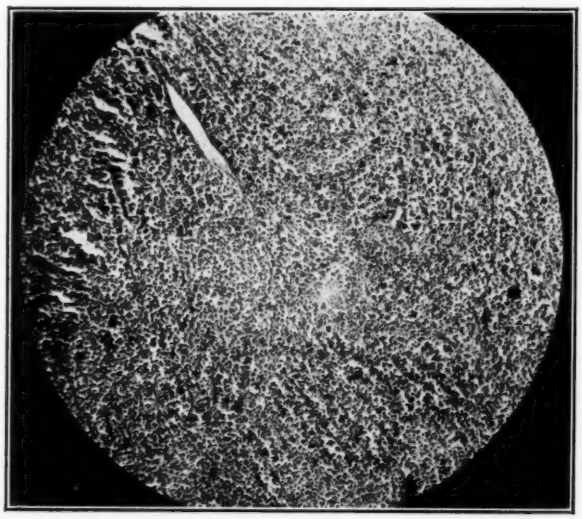

Fig. 4.—Section of lesion in stomach (low power), showing carcinomatous degeneration.

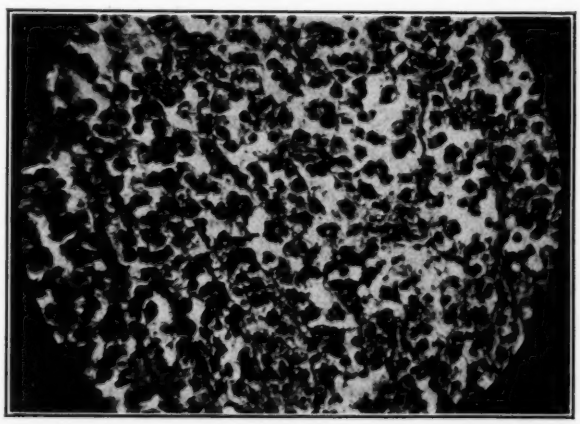

Fig. 5.—Section of carcinoma of the stomach (high power).

from involvement, except the lower segment of the sigmoid flexure where there was a large nodular mass infiltrating the wall but not obliterating the lumen.

Microscopic Studies.—Sections taken from various tumor masses showed similar changes. There was a marked anaplasia of the cells. The cells were epithelial in type with mitotic figures and hyperchromatic nuclei. There were areas also with extreme degeneration where it was impossible to make any differentiation of nuclear structures. They were faintly stained with eosin. The original tumor mass from the stomach (Figs. 4 and 5) did not show any tendency to acinar formation, but the tumor cells showed typical invasive characters

extending through the stroma which was dense fibrous tissue. Sections taken from the liver, kidney, and the rest of the organs showed areas of tumor cells, marked leucocytic infiltration, and occasional giant cell structure resembling that of foreign body giant cells. Sections of the spleen, lung, and pancreas did not show any evidence of metastatic involvement.

Anatomic Diagnosis.—Scirrhous carcinoma of the stomach with ulceration and perforation. Generalized peritonitis. Intraabdominal hemorrhage. Generalized metastatic carcinoma of the liver, kidney, lymphatics of omentum, and mesentery. Massive metastasis to the skin. Terminal edema of lungs.

COMMENT

Syphilis was present in several of the cases reported in the literature. In some of these cases it was thought at first that the nodules were of syphilitic origin. The biopsy findings, however, ruled with certainty against such an assumption in the case presented above.

The case was somewhat unusual because it presented no involvement of the lungs which are, as a rule, included in such metastatic processes. Furthermore, when skin metastases occur they usually are associated with adenocarcinoma of the stomach rather than with the scirrhous type of cancer.

With regard to the route of distribution of the cancer cells to the skin there are two views: the hematogenous and the lymphogenous. In favor of the hematogenous distribution of these metastases is the fact that the metastases involve various viscera, the pericardium, the suprarenal glands as well as the skin, and, furthermore, that they are frequently not associated with any regional adenopathy. A biopsy of the skin lesions frequently shows the presence of many blood vessels which are surrounded by cancer cells. The nodules may appear simultaneously. There may be no dilatation of the lymph vessels and lymph spaces in the skin, and there may be cancer cells in the blood vessels and capillaries of the metastatic skin nodule, as well as in the blood vessels of other organs involved in the malignant process. On the other hand, Vedel, Puech, and Chardonneau[12] take the opposite view. Likewise Guilbert[2] has never noticed a lesion involving the arterial wall or cancerous emboli to the interior of these vessels. In some of the sections studied by him he noticed the lymphatic vessels in the skin to be distended with neoplastic cells. He feels, therefore, that the dissemination must take place by the lymphatic route. It is likely, however, that these tumors reach the skin both by the lymphatic and hematogenous route.

SUMMARY AND CONCLUSION

1. A report of a case of carcinoma of the stomach with numerous metastases to the skin, and with complete necropsy findings is presented.

2. The diagnosis of the nature of the skin lesion depends on biopsy for corroboration.

3. This case is also unusual because (1) it presents no lung involvement, and (2) the metastasizing cancer was of the scirrhous type.

4. The evidence points to both the hematogenous and the lymphatic route as means of transmission of the tumor cells.

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1004 MAY BUILDING.

THE HYDROGEN ION CONCENTRATION OF NASAL SECRETION IN CHILDREN WITH ACUTE CORYZA*

A PRELIMINARY REPORT

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THE object of the following experiment was to determine the range or normal variation of the hydrogen ion concentration in the nasal secretion of children and note any change which may occur in individuals during an attack of acute coryza.

MATERIALS AND METHODS

The nasal secretions for these determinations were obtained from children presented for annual or semiannual physical examination. They were from three months to thirteen years of age and no discrimination was made with respect to sex. Those children in whom no pathologic conditions were detected, were considered as normal and the P_H of their respective nasal secretions was recorded as such.

The P_H determinations of nasal secretion in acute coryza were made early in the disease. The children on whom these determinations were made represent selected cases in so far as no other pathologic condition could be detected. It should be emphasized that the P_H determinations were made on the nasal

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secretion during the acute stage of the disease when the nasal discharge was thin, clear, and most profuse. In several instances P_H determinations were made on the same child, i.e., normal and during an attack of acute coryza.

With only slight modifications making it adaptable for nasal secretions, the methods used were the same as those developed by Clark.[1] The indicators used were phenol red having a P_H range of 6.7 to 8.2 and brom cresol purple from 5.6 to 6.9.

RESULTS

The results obtained from these experiments are presented in Tables I and II, and in a graphic record (Fig. 1) of an individual case whose nasal secretion was examined over a period of several weeks, during which time an attack of acute coryza developed.

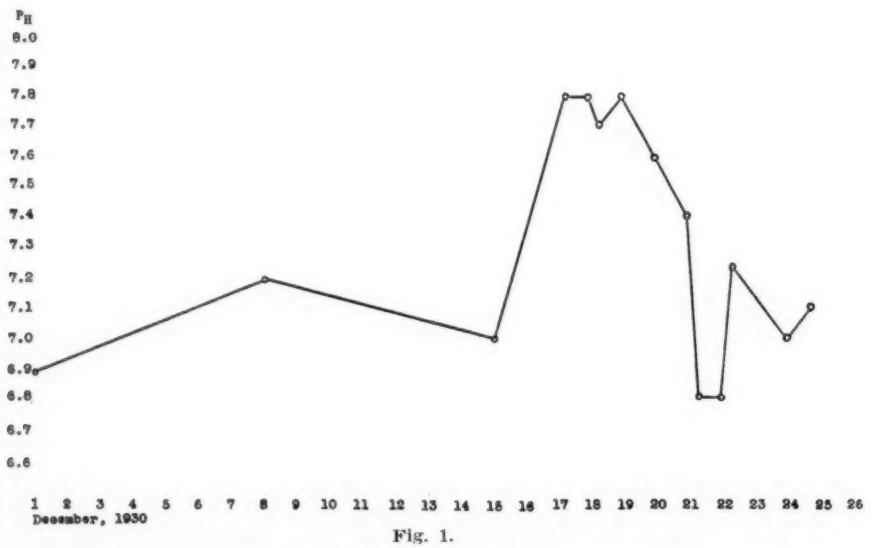

Fig. 1.

COMMENT AND CONCLUSIONS

The P_H values recorded in Table I were obtained from children found to be of normal development in every respect. It will be noted that the P_H of their respective nasal secretions range from 6.8 to 7.2 with a mean value of 7.03 ± 0.021. While in some instances these results are slightly acid and in others slightly alkaline, they are practically within the neutral range.

Acute coryza is usually ushered in with several paroxysms of sneezing followed by a marked congestion of the nasal mucous membranes. During this period of congestion, there is quite a profuse thin, clear, watery discharge from the nose which may continue for forty-eight hours or longer. The P_H determinations were made on this watery discharge and the results obtained are recorded in Table II.

The P_H range of the nasal secretion in children with acute coryza extends from 7.2 to 7.9 with a mean value of 7.69 ± 0.039. From these results, it may be stated that in the early stages of acute coryza the nasal secretion gives an alkaline reaction.

TABLE I

NORMAL

CASE	P_H	CASE	P_H
1	7.0	19	7.2
2	7.2	20	7.2
3	7.2	21	7.2
4	7.1	22	6.9
5	7.2	23	7.0
6	6.8	24	7.0
7	7.0	25	7.2
8	7.0	26	6.8
9	7.1	27	7.0
10	7.2	28	7.1
11	7.0	29	7.1
12	7.1	30	7.2
13	6.9	31	6.8
14	7.0	32	6.9
15	7.0	33	6.8
16	7.0	34	7.0
17	7.0	35	7.0
18	7.0		

Mean = 7.03 ± 0.022
St. D. = 0.126

TABLE II

ACUTE CORYZA

CASE	P_H	CASE	P_H
101	7.8	113	7.6
102	7.7	114	7.7
103	7.8	115	7.8
104	7.6	116	7.8
105	7.8	117	7.8
106	7.9	118	7.8
107	7.7	119	7.5
108	7.4	120	7.4
109	7.2	121	7.7
110	7.8	122	7.9
111	7.9	123	7.8
112	7.7	124	7.5

Mean = 7.69 ± 0.032
St. D. = 0.189

The curve presented in Fig. 1 represents the P_H or nasal secretion in a boy, aged eight years, who had been under observation for several weeks before the onset of an attack of acute coryza. In this case the normal P_H of the nasal secretion on December 1 was 6.9; on December 8, 7.2; and on December 15, 7.0. During the night of December 16 this child had several attacks of sneezing and the next morning, December 17, a slight nasal discharge was noted which gave a P_H value of 7.8. On the afternoon of the same day another determination was made and found to be the same, 7.8. The next morning, December 19, the P_H of the nasal secretion was 7.6, and on December 20, 7.4. The following day, December 21, the mucous membranes of the nose while quite congested, were dry, and it was with some difficulty that sufficient secretion could be obtained for the P_H determination. The results on this day were, A.M. 6.8 and P.M. 6.7. On December 22, the mucous membranes had a normal appearance and the nasal secretion gave a P_H value of 7.2; on December 23, the P_H was

7.0; and on December 24, 7.1. During the entire course of this attack the only therapeutic measures employed were complete rest in bed and plenty of fluids.

From these determinations it appears that the P_H of nasal secretion in normal children varies from 6.8 to 7.2 with a mean of 7.03 ± 0.021. These values are well within the neutral range of hydrogen ion concentrations. During the early stage of acute coryza, the P_H of nasal secretion extends from 7.2 to 7.9 with a mean of 7.69 ± 0.039. These results indicate an alkaline reaction.

SUMMARY

1. The hydrogen ion concentration of nasal secretion obtained from normal children extends from 6.8 to 7.2 with a mean of 7.02 ± 0.021. These results indicate practically a neutral reaction of the nasal secretion.

2. The hydrogen ion concentration of nasal secretion obtained from children during the early stages of acute coryza gave results extending from 7.2 to 7.9 with a mean of 7.69 ± 0.039. These P_H determinations indicate an alkaline reaction of the nasal secretion in this disease.

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106 EAST SIXTY-FIFTH STREET.

A CASE OF MARKED CREATINEMIA WITH RECOVERY*

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IT IS now well established that the retention of considerable amounts of creatinine is associated with renal disease, and that the quantity of creatinine found in the blood is a fairly accurate index of the degree of renal impairment. For some time following the observations of Myers, creatinine values over 5 milligrams per 100 c.c. were thought to denote an early fatal termination. Exceptions were not long in appearing and one of these, reported by Myers,[1] was a patient with ureteral obstruction from stones whose blood creatinine was 21.2 mg. With relief of the obstruction the creatinemia returned to normal and the patient recovered.

The following report deals with an instance of marked creatinemia with recovery.

CASE REPORT

A colored woman, aged twenty-eight years, was admitted to the Cleveland City Hospital on April 4, 1932, at 6 A.M., complaining of asthma. Her past history was negative. Her present difficulty went back four years. During this time she has had attacks of asthma at irregular intervals. These attacks were associated with colds which are always present when the attacks occur. The attack which brought the patient into the hospital was of two days'

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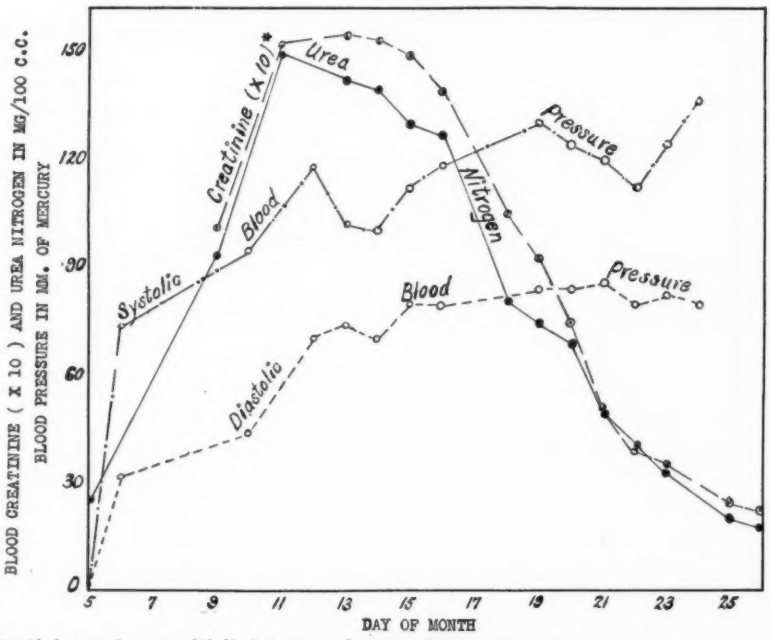
duration. Patient said she has had some sort of "spells" during which time she would fall asleep for two days without being able to be aroused. The first attack, five months previous to admission, came on after she had become extremely angry about something. Two days before admission the patient became frightened by a fire.

On physical examination the patient was found to be a well nourished and developed colored woman who presented the typical picture of an acute attack of asthma obstruction. The patient was thoroughly conscious but "felt as if she were going to die." The other positive findings were as follows: examination of lungs showed the typical findings of asthma. The surprising findings were that the patient was pulseless, the systolic and naturally the diastolic pressure were so low that neither could be measured by a mercury manometer. This finding was checked several times by several different men. One-half cubic centimeter of adrenalin was given, and within an hour the attack of asthma subsided and the patient said she felt better "all over." About four or five hours later, the patient had another feeling of impending death; she became restless and felt "very peculiar." She was then given the



*Creatinine values multiplied by ten for graphing purposes

Chart 1.

routine treatment for shock including hypertonic solutions of glucose intravenously, caffeine sodium benzoate, adrenalin, and normal saline solution intravenously. She felt better. During the entire period, the blood pressure could not be measured. At 10 P.M., or about sixteen hours after admission, her blood pressure was measured for the first time. It was 74/32. The first dose of adrenalin had caused the asthma to disappear.

The examination of the urine showed two plus albumin and the sediment was composed of hyaline and granular casts and a great many white blood cells. Urea nitrogen was 27 mg. per 100 c.c. Creatinine test was not made. The fluid intake on the day of admission was 2300 c.c., but the output was only 50 c.c. The red blood count, the white blood count, and hemoglobin estimation were of no significance.

There was a very rapid rise of both urea nitrogen and creatinine to 142 mg. and 15.4 mg. per 100 c.c. respectively on April 13. (Chart I.) From this time, there was a slower fall of these nitrogen elements of the blood; i.e., while the height was reached in about eight days, the return to normal required twelve days.

The blood pressure gradually rose but did not reach its highest point until April 20, or two weeks after admission.

The output gradually increased until on April 15, it totalled 1660 c.c. and contained 12.8 gm. of urea or about one-half the normal amount. The creatinine in the blood was just receding from its high point. On April 26, the output was 3100 c.c., the urea output was 21.7 gm., the urea nitrogen and creatinine were 18 mg. and 2.3 mg. respectively.

The phenolsulphonephthalein output was zero on April 12. This was not repeated. The urine gradually cleared, and became negative.

The CO_2 determinations varied from 43 to 64 volumes per cent.

Comment.—The amazing fact about the course of this case is that in spite of the blood and other laboratory findings the patient was perfectly comfortable and conscious during the entire time of her hospital stay.

The exact cause of the findings is not known; however, it is suggested that due to the marked drop of blood pressure and the resultant disturbance in the blood flow through the kidneys, there was an accumulation of nitrogen metabolites in the blood together with the other evidences of kidney insufficiency, and as the blood pressure rose to normal, all the evidences of kidney insufficiency disappeared. Whether the fall in blood pressure was due to an anaphylaxis as the result of the asthma obstruction is difficult to say.

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7016 Euclid Avenue.

THE NORMAL RANGE OF CALCIUM AND INORGANIC PHOSPHORUS IN THE SERUM OF HEALTHY NONPREGNANT WOMEN*†

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IN A PREVIOUS publication the authors[1] presented evidence, both from the literature and from their own work, that the generally accepted range of 9 to 11 mg. Ca per 100 ml. of serum is too low. The present study, in which the calcium and phosphorus was determined in 207 healthy nonpregnant women, fully confirms our earlier conclusions.

The same methods were employed as before. The calcium was determined by the method of Kramer and Tisdall[2] with slight modifications in technic as suggested by Collip and Clark,[3] Clark,[4] and our own experience. Tests were run from time to time throughout the course of the study by making multiple determinations on the same sample of serum, by recovering calcium added to the serum, and by determining the calcium in prepared solutions of known strength. These tests indicate an average error of from 1 to 2 per cent.

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†From the Laboratory of the Maternity Hospital and the Department of Obstetrics, School of Medicine, Western Reserve University.

Phosphorus was determined by the method of Kuttner and Cohen[5] as modified by Kuttner and Lichtenstein,[6] with some suggestions in handling from Dr. Shohl.[7] Standards of different strength were used so that unknowns could always be read within 3 mm. plus or minus of the standard used, set at 20 mm. Tests as described above indicate an average error of 5 to 6 per cent.

The subjects were nurses, hospital employees, and patients who came to our prenatal clinics, but were found not to be pregnant. Blood was drawn just before lunch in all cases except for the clinic patients, where it was taken just after lunch. It was drawn into centrifuge tubes, allowed to clot, and then

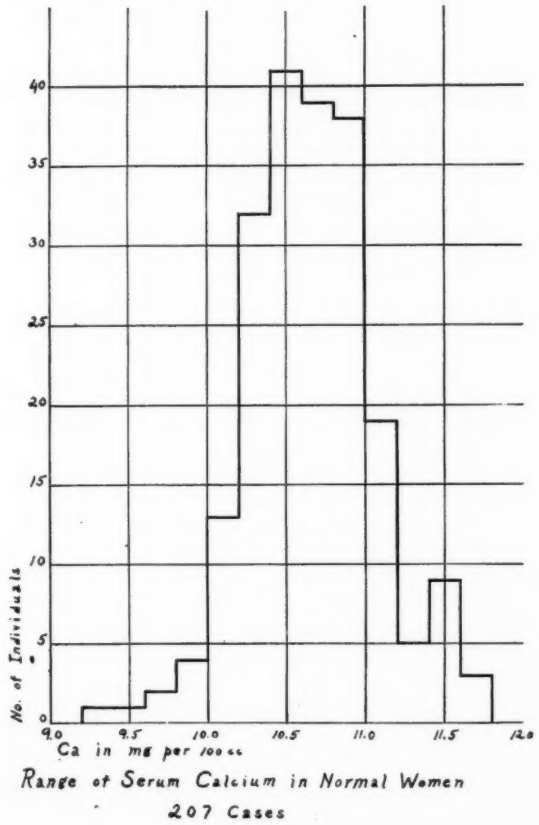

Fig. 1.

centrifuged. The serum was poured off, recentrifuged to remove all red cells, and used for the determinations.

In Table I the subjects have been grouped first on the basis of diet, those eating in the dormitory having a more or less standard diet, those at home eating a freely selected diet, and the outpatients being on practically a minimum diet. As regards calcium, the greatest difference in the averages of these three groups is 0.17 mg., while the probable error of this difference is 0.064 mg. In some cases this difference may be considered as significant, but we do not feel justified in so considering it here, where the analyses are made in biologic fluids.

The subjects were then regrouped on the basis of age into those below thirty years of age and those thirty or over. The selection of thirty was made

TABLE I

CALCIUM AND INORGANIC PHOSPHORUS IN THE SERUM OF NORMAL WOMEN

GROUP	CALCIUM				PHOSPHORUS			
	NO. IN GROUP	AVERAGE	PROBABLE ERROR OF THE MEAN	PROBABLE ERROR OF THE DIFFERENCE	NO. IN GROUP	AVERAGE	PROBABLE ERROR OF THE MEAN	PROBABLE ERROR OF THE DIFFERENCE
		(mg./100 c.c.)	(mg./100 c.c.)			(mg./100 c.c.)	(mg./100 c.c.)	
Dormitory diet	134	10.63	0.025		132	3.74	0.022	
Independent diet	31	10.58	0.047		30	3.36	0.063	
Outpatients	42	10.75	0.040		41	3.40	0.044	
Greatest difference		0.17		0.064		0.38		0.063
Below 30 years—Nurses	142	10.70	0.021		109	3.84	0.021	0.054
Outpatients					32	3.48	0.049	
Thirty years and over	65	10.54	0.038		62	3.34	0.041	
Difference		0.16		0.045		0.50		0.044
Hospital diet								
Below 30 years					97	3.82	0.022	
Thirty years and over					35	3.51	0.050	
Difference						0.31		0.054
Independent diet								
Below 30 years (nurses)					12	3.72	0.058	
Thirty years and over					27	3.13	0.058	
Difference						0.59		0.085
Below 30 years								
Hospital diet					97	3.82	0.022	
Independent diet					12	3.72	0.058	
Difference						0.10		0.063
Thirty years and over								
Hospital diet					35	3.51	0.050	
Independent diet					27	3.13	0.058	
Difference						0.38		0.078

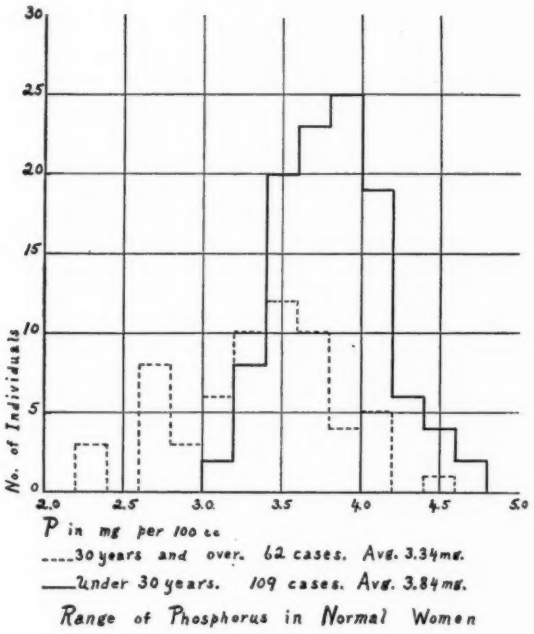

Fig. 2.

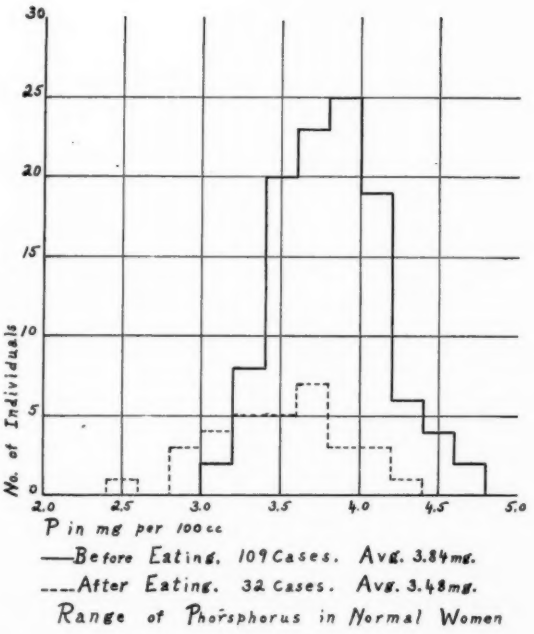

Fig. 3.

because it was the only significant point found when our subjects were grouped in ten year periods. The average difference in this case is 0.16 mg. with a probable error of 0.045 mg. Again we do not feel justified in calling this a significant difference. From this we conclude that calcium is not materially affected by age or diet, so long as there is an adequate intake.

With phosphorus, however, a glance at Table I shows marked differences. On the first diet grouping the greatest difference of the averages given is 0.38 mg., while the probable error is only 0.063 mg. This difference is unquestionably significant. The age grouping brings out another factor, those under thirty are separated into nurses and outpatients, with widely differing averages. This is due to the fact that the former were taken before lunch and the latter after. It has been shown by Bollinger and Hartman,[8] Harrop and Benedict,[9] and others that there is a marked fall in the inorganic phosphorus of the blood dur-

TABLE II

CALCIUM AND INORGANIC PHOSPHORUS IN THE SERUM OF NORMAL NONPREGNANT WOMEN
OUTPATIENT GROUP

ALL FIGURES GIVEN IN MG. PER 100 ML. SERUM

NAME	AGE	PARA	DATE	CA	P
L. W.	30	i	5–27–31	10.22	3.75
			6–17–31	11.41	4.35
			8–12–31	10.68	2.72
			Avg.	10.77	3.61
A. H.	29	i	8– 3–31	10.56	3.10
			9–14–31	11.23	3.60
			Avg.	10.90	3.35
R. G.	19	i	4–24–31	10.91	2.53
			5–15–31	10.84	3.10
			6– 5–31	10.60	2.83
			Avg.	10.78	2.82
I. A.	31	ii	9– 9–31	11.26	—
			9–30–31	11.55	3.53
			Avg.	11.40	3.53
A. C.	31	ii	10–24–31	11.04	2.83
			11–21–31	10.37	3.77
			Avg.	10.70	3.30
L. D.	24	i	10–11–30	10.95	3.84
			12–20–30	10.17	3.41
			3–24–31	10.25	2.53
			Avg.	10.46	3.26
M. H.	23	i	10–28–30	10.16	—
			11–25–30	10.47	3.86
			12–23–30	10.72	4.17
			Avg.	10.78	4.01
N. D. L.	22	i	12–15–31	11.14	4.04
			1–12–32	11.07	3.31
			Avg.	11.10	3.67
R. W.	19	i	10–18–30	10.78	3.57
			12–13–30	10.52	2.93
			Avg.	10.65	3.25
N. W.	22	i	9–29–31	11.00	2.77
			10–20–31	11.11	3.51
			11– 3–31	10.74	—
			Avg.	10.95	3.14
L. A.	16	i	3– 5–32	10.42	4.02
			3–26–32	10.73	4.17
			Avg.	10.58	4.10
O. P.	19	ii	11–13–31	10.71	3.29
			1–22–32	10.00	3.76
			Avg.	10.35	3.52
M. G.	27	i	5– 5–31	10.94	3.11
			5–26–31	10.86	2.91
			6–16–31	10.73	2.93
			8–18–31	10.68	2.72
			8–29–31	—	2.36
			Avg.	10.80	2.81

Maximum deviation from the individual average Ca 0.64 mg. P 0.88 mg.
Average deviation from the individual average Ca 0.25 mg. P 0.38 mg.

ing carbohydrate metabolism. These groups, therefore, are not comparable. Those taken after eating cannot be considered here, and are not included in the subsequent groupings.

Between the compared age groups we find a difference in the averages of 0.50 mg., and a probable error of 0.044 mg. Here again is a significant difference. Either diet or age causes a difference in the serum phosphorus. In order to eliminate one of these two factors, those eating the standard hospital diet and those eating independently at home are both subdivided on the basis of age, and

in each case the difference found between the averages is significant. Then those below thirty are grouped by diet, and the difference is nominal. This seems to indicate that age is the important factor. The last group, that over thirty divided by diet, does not fall into line as expected. This failure may be due to a number of reasons, but the most probable seems to be that of individual variation. If age is the affecting cause, it is entirely probable that such a change would come not at any fixed year, as at thirty, but would vary with each subject

TABLE III

CALCIUM AND INORGANIC PHOSPHORUS IN THE SERUM OF NORMAL NONPREGNANT WOMEN HOSPITAL GROUP

ALL FIGURES GIVEN IN MG. PER 100 ML. SERUM

NAME	AGE	DATE	CA	P	NAME	AGE	DATE	CA	P
M. L.	38	6–18–31	10.34	3.16	M. M.	21	4–23–31	10.00	3.37
		10– 1–31	10.50	3.27			2– 3–32	10.18	3.87
		Avg.	10.42	3.21			Avg.	10.09	3.62
B.	38	2–12–31	10.17	4.06	F. B.	26	8–20–31	10.58	4.31
		2–11–32	9.92	3.44			2–11–32	9.80	4.03
		Avg.	10.04	3.75			Avg.	10.19	4.17
B. F.	32	4–16–31	10.87	3.41	K.	38	2– 5–31	9.33	3.19
		2–11–32	10.08	3.47			2–11–32	9.87	2.76
		Avg.	10.47	3.44			Avg.	9.60	2.97
D.	43	2–12–31	9.90	3.66	J. M.	28	9–10–31	10.75	3.50
		2–11–31	——	3.82			2–17–32	9.60	4.40
		Avg.	9.90	3.74			Avg.	10.17	3.95
N. C.	25	5–21–31	10.57	4.10	W. P.	26	10–29–31	11.53	3.31
		2–17–32	9.55	3.69			2–18–32	9.70	3.49
		Avg.	10.06	3.89			Avg.	10.61	3.40
M. L.	28	8–14–31	10.52	4.00	M. W.	32	8–17–31	10.82	2.64
		2–18–32	9.90	4.58			1–18–32	9.87	2.44
		Avg.	10.21	4.29			Avg.	10.34	2.54
C. A.	26	6–11–31	11.06	3.86	N.M.	24	3– 3–32	9.94	4.18
		9–10–31	10.98	3.66			4– 7–32	9.80	3.76
		2–22–32	9.99	3.76			Avg.	9.87	3.97
		Avg.	10.68	3.76					

Maximum deviation from the average Ca 0.92 mg. P 0.45 mg.
Average deviation from the average Ca 0.37 mg. P 0.18 mg.

studied. It would not be unlikely, therefore, to find disagreement in this group. We feel that the evidence favors the age factor, and that there is a decline in the phosphorus content of the serum of women, taking place, on the average, at around thirty years of age.

On the basis of the findings presented in Table I we have included all the 207 cases in the distribution curve given in Fig. 1. Over two-thirds, 81 per cent, fall within plus or minus 0.4 mg. of the average, 10.6 mg. per 100 ml. 94 per cent fall between 10.0 and 11.5 mg. On the basis of this evidence we claim that the normal range of serum calcium in healthy women, as found in Cleveland, is from 10.0 to 11.5 mg. per 100 ml. of serum.

In Fig. 2 we have given the distribution curve for phosphorus for those thirty years and over, and for those under thirty. Over two-thirds of the latter, 80 per cent, fall within plus or minus 0.4 mg. of the average, 3.8 mg. per 100 ml. Ninety-two per cent fall between 3.2 mg. and 4.4 mg., which may be considered the normal range for inorganic phosphorus for healthy women under thirty. The curve for those over thirty is, as would be expected, much less regular. The average found was 3.3 mg., and the range from 2.6 mg. to 4.2 mg. per 100 ml.

Fig. 3 shows the difference due to carbohydrate metabolism. The 109 cases are the same as shown in Fig. 2, under thirty, while the 32 cases are from the outpatient clinic, where the samples were drawn after lunch. There is a distinct difference in the averages and in the curves.

Tables II and III are given to confirm the validity of our findings. These tables show repeated tests on the same individual at intervals varying from two weeks to a year. They show a remarkable constancy in the calcium and phosphorus levels of a given individual. In Table II, outpatients, the maximum deviation of any finding from the average for that individual was only 0.64 mg. for calcium and 0.88 mg. for phosphorus, while the average deviation of any finding from the individual average was 0.25 mg. for calcium and 0.38 mg. for phosphorus.

In Table III, hospital group, there are some larger variations. It will be noticed that these occur between tests made during the early months of the year, our winter months, and those made during the summer or fall months. This led to the belief that there might be a seasonal variation in calcium. The evidence, however, proved contradictory and no such conclusion can be justified on the basis of our studies. No evidence was found of any seasonal variation in phosphorus.

SUMMARY

The calcium content of the serum of normal adult women is not affected by age or diet, provided there is an adequate intake.

The normal range of serum calcium in healthy women, under conditions in Cleveland, is from 10 to 11.5 mg. per 100 ml. of serum.

The inorganic phosphorus of the serum of healthy women is subject to an age variation, the normal range in women over thirty being lower than that for women under thirty.

Three and two-tenths to 4.4 mg. per 100 ml. of serum is the normal range of inorganic phosphorus for women under thirty, when the blood is drawn before eating.

The range for those over thirty is found to be from 2.6 to 4.2 mg. per 100 ml. of serum.

No conclusive evidence of seasonal variation was found in either the calcium or the inorganic phosphorus.

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STUDIES ON GASTRIC MUCIN*

Its Effect on Gastric Acidity

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THE physiologic rôle of gastric mucus has in the past attracted relatively little attention. That it possesses a protective function was suggested by William Beaumont[1] in 1833 and reiterated by Claude Bernard,[2] Schiff,[3] Pavlov,[4] Klug,[5] and more recently by Whitlow.[6] Heidenhain[7] in 1879 ascribed to it the power of reducing gastric acidity. Pavlov[4] stated that "a perfectly pure juice may have its acidity reduced to the extent of 25 per cent by neutralization with mucus." Gies[8] in 1903 concluded that whereas precipitated mucoid shows practically no acid-combining power, "in the hydration of mucoid by pepsin-acid . . . . acid combines with the dissolved proteid products formed in the process." Extending the work of Gies, Foster[9] in 1907 observed that gastric mucus obtained by lightly scraping the mucosa of pigs' stomach, in the presence of pepsin is capable of combining with hydrochloric acid to a relatively marked degree. Further, Lim[10] noted that the concentration of mucus was higher than normal when the rates of gastric secretion (HCl output) were low. Because of its power to combine readily with free acid, its "protective, soothing lubricating rôle" and the fact that "its secretion or ingestion causes no chemical disturbance in the body and no unfavorable effect on gastro-intestinal secretory or motor activity," it was recently proposed by Fogelson[11] as an ideal antacid. Using Pavlov pouch dogs, Fogelson[11] found that gastric mucin, a substance prepared from the mucosa of hogs' stomach, has a high acid combining power. When administered to patients with chronic peptic ulcer, it resulted in complete relief of symptoms within three days of treatment. Kim and Ivy[12] later reported the prevention of ulcer formation in 17 dogs with biliary fistula by feeding 15 gm. of mucin twice daily.

*From the Medical Service of the Michael Reese Hospital.
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Pursuant upon Fogelson's interesting preliminary report, we undertook a comprehensive study of its clinical value and at this time, wish to report the effect of commercial mucin and some of its fractions upon the gastric acidity in eight patients with uncomplicated chronic peptic ulcer. Our results in the treatment of 28 patients are to be reported elsewhere.[12a] The mucin preparations and the information pertaining thereto were furnished by Dr. David Klein, of The Wilson Laboratories.

GENERAL PROCEDURE

A Rehfuss tube was passed into the fasting stomach and retained throughout the period of observation. The stomach was emptied, the test meal given and at fifteen minute intervals 10 c.c. of gastric contents were aspirated and examined immediately for free and total acidity. At the termination of each aspiration, the residual content of the tube was forced back into the stomach and the end of the tube clamped. Individual series of observations were made on successive mornings.

1. ALTERATIONS IN THE ACIDITY FOLLOWING ONE OR MORE BLAND FEEDINGS WITH AND WITHOUT GASTRIC MUCIN

With the tube in situ, gastric secretion was stimulated by having the patient drink 400 c.c. of water. One hour later a bland breakfast (qualitatively and quantitatively the same throughout the period of study) was served. Maintaining the usual ward ulcer routine, an interval feeding of three ounces of custard was given approximately two hours later. With one or more such series established as controls, one-half ounce of gastric mucin was added to the breakfast and to the custard. We hoped thereby to determine to what degree the buffering action of a bland feeding is enhanced by the addition of this amount, the usual therapeutic dose, of mucin. Such studies were carried out upon three patients.

In patient 1S (Table I, Chart 1), the control breakfast on two occasions resulted in a complete absence of free HCl for one-half hour. By the addition of mucin, a similar result was obtained in one experiment; in another, free HCl was absent for one-fourth of an hour. The subsequent addition of custard had no effect in one control, but in the other there was an absence of free HCl for one-fourth of an hour. Mucin added to custard produced an absence of free acid for one-fourth and one-half hour respectively in two experiments. A mild or moderate secondary hyperstimulation was observed in three of four instances in which mucin was used.

In patient 2H (Table II, Chart 2), the results with mucin were much more striking. Whereas the control breakfast produced an absence of free HCl for one-fourth of an hour, with mucin no free acid was detected for one hour in one experiment and for one and one-half hours in two subsequent ones. In the latter two experiments a secondary hyperstimulation rapidly ensued. The later addition of custard to the control reduced the free HCl to a moderate degree, but custard together with mucin reduced the free acidity to zero for one-half hour, although at the three-quarter hour period its value was the same as the control.

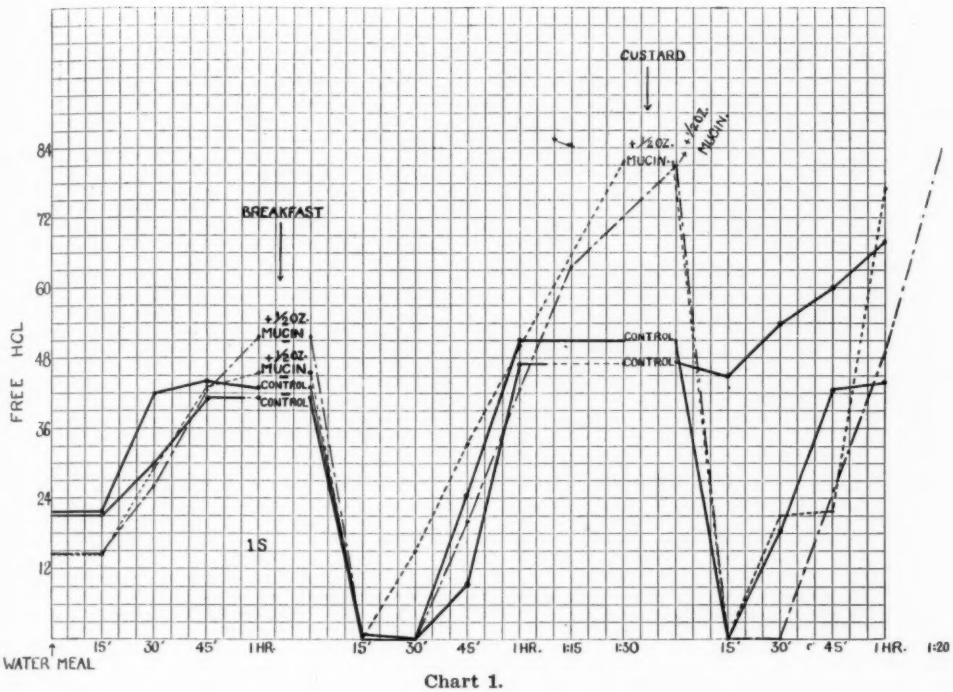

Chart 1.

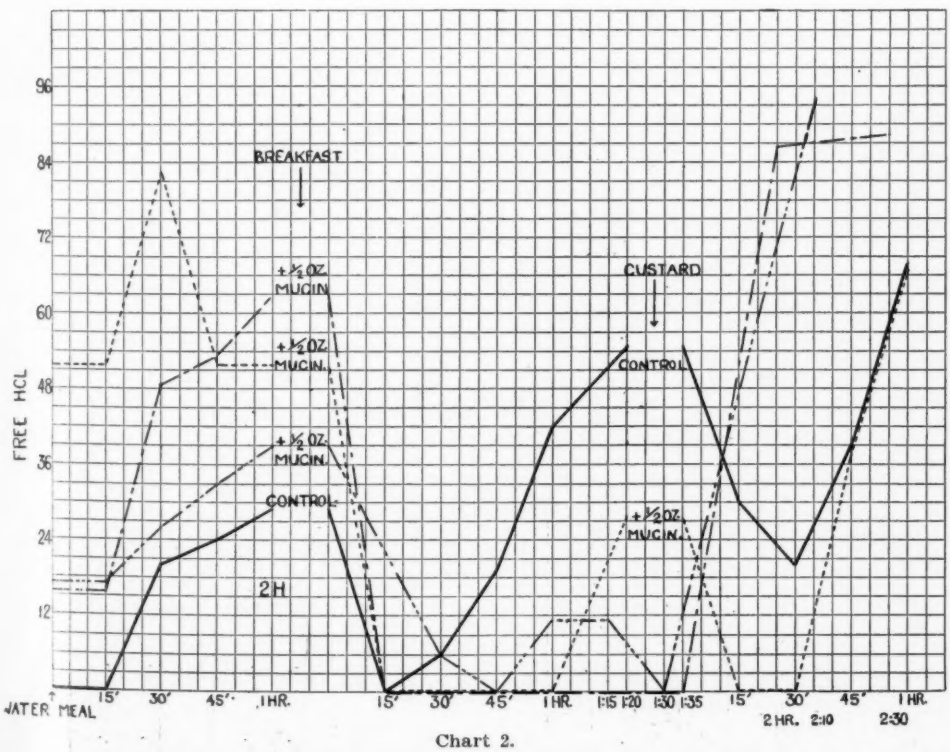

Chart 2.

Charts 1 and 2.—Gastric free acidity following one or more bland feedings with and without gastric mucin. Gastric secretion was stimulated by 400 c.c. water and breakfast given one hour later. Custard, if used, was given two hours after breakfast (Patients 1S and 2H).

TABLE I

GASTRIC ACIDITY FOLLOWING ONE OR MORE BLAND FEEDINGS WITH AND WITHOUT GASTRIC MUCIN

(PATIENT 1S)

TEST MEAL		¼ HR.	½ HR.	¾ HR.	1 HR.	FEEDING	¼ HR.	½ HR.	¾ HR.	1 HR.	1¼ HR.	1½ HR.	1¾ HR.	FEEDING	¼ HR.	½ HR.	¾ HR.	1 HR.	1¼ HR.
400 c.c. Water	*FA	21.7	42.0	44.0	43.0	Breakfast	1.0	0.0	9.5	47.0			---	Custard	45.0	54.0	60.0	68.0	
(Control)	*TA	26.7	48.0	50.0	50.0	(Control)	34.0	44.5	45.0	77.5	---	---		(Control)	69.0	78.0	82.5	88.0	
400 c.c. Water	FA	21.0	30.0	41.0		Breakfast	0.0	0.0	27.5	51.0		51.0	---	Custard	0.0	18.5	43.0	44.0	
(Control)	TA	26.0	34.0	45.0	---	(Control)	30.0	47.0	71.0	82.5	---	87.0	---	(Control)	48.0	75.5	91.0	87.0	
400 c.c. Water	FA	14.5	26.0	42.5	52.0	Breakfast	0.0	0.0	20.0	43.0	64.0		81.0	Custard	0.0	0.0	---	49.0	84.0
	TA	16.5	29.5	45.5	56.5	+½ oz. mucin	61.0	62.0	52.0	74.0	91.0	---	97.0	+½ oz. mucin	?	82.0	---	89.0	113.0
400 c.c. Water	FA	14.5	29.0	43.0	45.5	Breakfast	0.0	15.0	33.3	50.5		82.0	---	Custard	0.0	21.0	22.0	77.0	
	TA	19.0	32.5	48.0	51.0	+½ oz. mucin	47.0	40.7	62.8	70.0	---	98.5		+½ oz. mucin	68.6	82.0	92.0	99.0	

*FA—Free acidity. *TA—Total acidity.

TABLE II

GASTRIC ACIDITY FOLLOWING ONE OR MORE BLAND FEEDINGS WITH AND WITHOUT GASTRIC MUCIN

(PATIENT 2H)

TEST MEAL		¼ HR.	½ HR.	¾ HR.	1 HR.	FEEDING	¼ HR.	½ HR.	¾ HR.	1 HR.	1 HR. 20 MIN.	FEEDING	¼ HR.	½ HR.	¾ HR.	1 HR.
400 c.c. Water	FA	0.0	20.0	24.0	29.0	Breakfast	0.0	6.0	19.0	42.0	55.0	Custard	30.0	20.0	39.0	68.1
(Control)	TA	5.0	24.0	28.0	33.0	(Control)	24.0	39.0	55.0	70.0	91.6	(Control)	74.0	68.8	87.5	96.9
400 c.c. Water	FA	52.0	82.5	52.0	52.0	Breakfast	0.0	0.0	0.0	0.0	28.0	Custard	0.0	0.0	38.0	67.0
	TA	57.0	86.5	56.0	58.0	+½ oz. mucin	51.7	50.0	50.0	76.6	94.0	+½ oz. mucin	71.2	77.5	98.0	112.0

												1½ HR.	1¾ HR.	2 HR.	2 HR. 10 MIN.	2½ HR.
400 c.c. Water	FA	16.0	49.0	53.3	63.0	Breakfast	0.0	0.0	0.0	0.0		0.0	---	86.6	---	88.3
	TA	18.0	51.0	57.5	69.0	+½ oz. mucin	50.0	60.0	63.0	62.5	---	84.0		116.6		108.3
400 c.c. Water	FA	17.0	26.0	33.0	39.0	Breakfast	---	6.0	0.0	11.4	11.3	0.0	---	---	94.3	---
	TA	20.0	29.0	36.0	43.0	+½ oz. mucin	---	66.0	83.0	84.3	100.0	103.0			117.0	

TABLE III

GASTRIC ACIDITY FOLLOWING ONE OR MORE BLAND FEEDINGS WITH AND WITHOUT GASTRIC MUCIN

(PATIENT 3M)

TEST MEAL		¼ HR.	½ HR.	¾ HR.	1 HR.	FEEDING	¼ HR.	½ HR.	¾ HR.	1 HR.	1¼ HR.	1½ HR.	FEEDING	¼ HR.	½ HR.	¾ HR.	1 HR.
400 c.c. Water (Control)	FA	38.0	46.0	47.0	57.5	(Control)	0.0	12.0	19.0	35.0		33.3	(Control)	28.6	27.5	31.0	44.0
	TA	41.5	50.0	51.0	62.5	Breakfast	45.0	57.0	63.0	72.0	---	63.3	Custard	73.6	76.2	77.7	83.0
														1 HR. 40 MIN.	2 HR.	2¼ HR.	2½ HR.
400 c.c. Water	FA	16.0	24.0	28.0	30.0	Breakfast	0.0	0.0	0.0	3.3		16.6		30.0	36.6	---	46.3
	TA	18.0	25.5	30.0	32.0	+½ oz. mucin	41.0	48.0	70.8	80.0	---	86.6		94.0	91.6		85.0

In patient 3M (Table III), we endeavored to compare the effects of two feedings (breakfast and later custard) with a similar breakfast containing one-half ounce of mucin (no custard). The results were interesting, for while the mucin produced an achlorhydria for three-fourths of an hour compared with one-fourth of an hour for the control, the final readings two and one-half hours after each breakfast were the same. No secondary hyperstimulation occurred.

2. COMPARATIVE INFLUENCE OF COMMERCIAL MUCIN AND "PURE" MUCIN

One brand of commercial mucin, according to the manufacturer, contains approximately 60 per cent "pure" mucin and 40 per cent peptone and ash

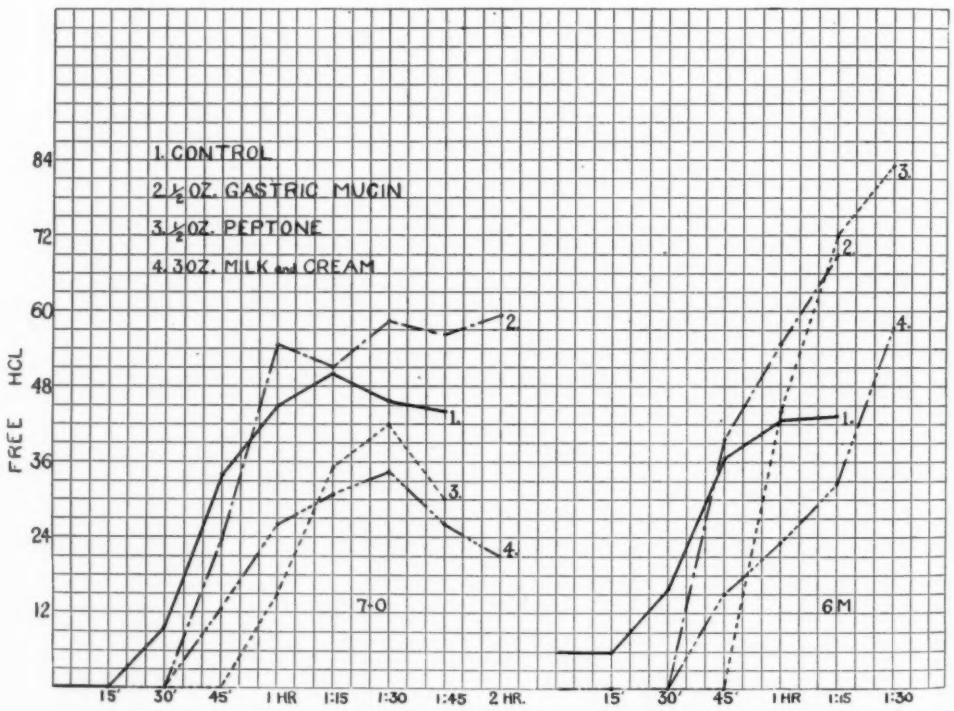

Chart 3.—Comparative effect of gastric mucin, peptone-phosphate fraction and milk and cream mixture. 500 c.c. oatmeal gruel was used as control and as gastric stimulant (Patients 7O and 6M).

material. It was desirable, therefore, to compare the relative antacid properties of commercial mucin ("gastric" mucin) with that of the "pure" mucin. Five hundred cubic centimeters of warm oatmeal gruel were used as the standard test meal (Crohn and Reis[13]). Accepting the response to such a meal as our control, the respective mucin preparation was added; in all instances, aspirations were continued until the stomach was empty. Observations were made upon two patients (Tables IV and V, Chart 3).

In both individuals one-half ounce of gastric mucin effected an absence of free HCl which persisted for one-half hour. "Pure" mucin produced the same result in patient 4B, but in patient 5M, the achlorhydria lasted for only one-fourth of an hour. To observe the effect of a larger dose, one ounce

TABLE IV

COMPARATIVE EFFECT OF GASTRIC MUCIN, PURE MUCIN, PEPTONE AND MILK AND CREAM UPON GASTRIC ACIDITY

(PATIENT 4B)

TEST MEAL		¼ HR.	½ HR.	¾ HR.	1 HR.	1¼ HR.	1½ HR.	1¾ HR.	2 HR.	2¼ HR.
Oatmeal gruel	FA	2.5	13.3	21.0	45.0	56.0	56.0	---	64.0	64.0
(Control)	TA	12.0	24.4	37.0	57.0	69.0	69.0		77.0	76.0
Oatmeal gruel	FA	0.0	0.0	15.5	46.2	59.0	72.5	92.0	Empty	
+ ½ oz. gastric mucin	TA	20.5	32.0	62.5	82.2	92.2	102.5	107.5		
Oatmeal gruel	FA	0.0	0.0	13.0	53.0	73.5	78.0	82.0	80.5	101.2
+ ½ oz. pure mucin	TA	19.5	35.0	53.0	82.5	99.5	100.0	103.0	99.0	118.7
Oatmeal gruel	FA	0.0	0.0	0.0	0.0	31.5	52.0	84.5	90.0	
+ 1 oz. gastric mucin	TA	41.0	45.0	60.0	79.0	101.5	119.0	118.0	116.0	
Oatmeal gruel	FA	0.0	0.0	2.0	34.0	45.0	69.0	81.0	78.7	
+ 1 oz. pure mucin	TA	34.5	51.0	67.0	95.0	93.0	105.0	104.4	98.7	
Oatmeal gruel	FA	0.0	0.0	0.0	0.0	29.5	55.5	69.5	87.0	
+ ½ oz. peptone	TA	30.0	35.0	52.0	74.0	90.5	94.4	108.0	110.0	
Oatmeal gruel	FA	0.0	0.0	0.0	16.0	30.0	45.0	70.0	66.5	
+ 3 oz. milk and cream	TA	16.0	21.0	43.5	56.0	77.0	73.0	92.5	98.0	
Oatmeal gruel	FA	0.0	10.0	29.0	36.6	38.0	51.7	34.5	44.0	
(Control)	TA	13.0	34.0	53.0	61.1	62.0	73.3	55.0	65.0	

TABLE V

EFFECT OF GASTRIC MUCIN AND PURE MUCIN UPON GASTRIC ACIDITY

(PATIENT 5M)

TEST MEAL		¼ HR.	½ HR.	¾ HR.	1 HR.	1¼ HR.	1½ HR.	1¾ HR.
Oatmeal gruel	FA	5.5	41.0	58.0	43.5	41.0	Empty	
(Control)	TA	22.0	53.0	70.0	56.0	59.0		
Oatmeal gruel	FA	0.0	0.0	12.0	48.9	49.0	43.7	60.6
+ ½ oz. gastric mucin	TA	21.0	35.0	62.0	78.9	74.0	68.8	79.4
Oatmeal gruel	FA	0.0	31.0	31.0	55.6	55.0	22.0	51.2
+ ½ oz. pure mucin	TA	27.5	55.0	34.0	74.4	73.0	50.0	70.6

TABLE VI

COMPARATIVE EFFECTS OF GASTRIC MUCIN, PEPTONE AND MILK AND CREAM UPON GASTRIC ACIDITY

(PATIENT 6M)

TEST MEAL		¼ HR.	½ HR.	¾ HR.	1 HR.	1¼ HR.	1½ HR.
Oatmeal gruel	FA	6.0	16.0	37.0	43.0	43.6	Empty
(Control)	TA	27.0	40.0	62.0	72.0	64.0	
Oatmeal gruel	FA	0.0	0.0	40.0	55.0	69.0	Empty
+ ½ oz. gastric mucin	TA	46.0	38.0	89.0	102.0	91.0	
Oatmeal gruel	FA	0.0	0.0	0.0	44.0	72.0	83.0
+ ½ oz. peptone	TA	51.0	58.0	73.5	84.0	94.0	97.0
Oatmeal gruel	FA	0.0	0.0	15.0	23.0	32.5	55.7
+ 3 oz. milk and cream	TA	31.0	29.0	42.0	50.0	58.5	70.0

each of gastric mucin and of "pure" mucin was given on successive days to patient 4B (Table IV). An achlorhydria lasting practically three-fourths of an hour was obtained with the "pure" mucin, contrasted with one hour when gastric mucin was administered. In all of these experiments, the achlorhydric period was superseded by a rapid rise in free HCl, the readings becoming equal to the control at the end of one hour when one-half ounce of either form of mucin was added and at the end of one and one-half hours when one ounce was given. A secondary hyperstimulation occurred in all mucin studies on patient 4B.

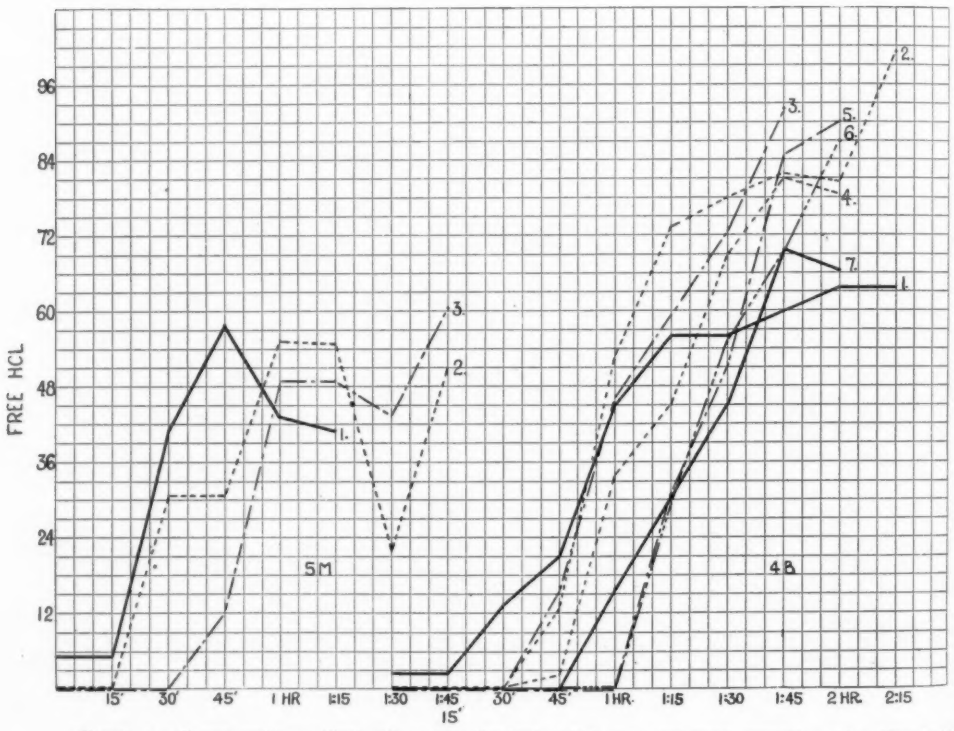

Chart 4—Comparative effect of: (1) Control (500 c.c. oatmeal gruel). (2) One-half ounce pure mucin. (3) One-half ounce gastric mucin. (4) One ounce pure mucin. (5) One ounce gastric mucin. (6) One-half ounce peptone-phosphate fraction. (7) Three ounces milk and cream mixture. The substances tested were added to 500 c.c. oatmeal gruel (Patients 5M and 4B).

3. NEUTRALIZING CAPACITY OF THE PEPTONE FRACTION

This phase of our study was conducted upon three patients in a manner similar to Part 2. One-half ounce of the peptone fraction was compared with one-half ounce of gastric mucin (Tables IV, VI, VII, Charts 3 and 4).

When the peptone fraction was added to the test meal, an absence of free HCl was noted for three-fourths of an hour in two patients (6M and 7O and for one hour in the third (4B) as compared with one-half hour when gastric mucin was tested. In the third patient (4B) the response to one-half ounce of peptone corresponded to that of one ounce of gastric mucin. The subsequent rapid appearance of free HCl previously remarked upon, was likewise observed in this group. Mild hyperstimulation was present in two patients (4B, 6M).

4. THE ACID COMBINING POWER OF A MILK AND CREAM MIXTURE. A COMPARISON WITH GASTRIC MUCIN AND ITS DERIVATIVES

The uniformity with which frequent milk and cream feedings bring about a rapid remission in many instances of uncomplicated peptic ulcer, as well as its recognized capacity for combining with free HCl, suggested a comparative study with gastric mucin and its fractions. Three ounces of milk and cream (equal parts) and one-half ounce each of gastric mucin, pure mucin, and peptone were tested in four patients. The method employed was the same as that in Parts 2 and 3 (Tables IV, VI, VII, VIII, Charts 3 and 4).

A milk and cream mixture of an amount equal to that used in routine ulcer management produced a total absence of free acid for one-half hour in three patients and for three-fourths of an hour in one. In three instances (6M, 7O, 8B) the readings at corresponding intervals were persistently less than their controls throughout the entire period of observation; in the fourth case they were less than the control for one hour. The rise in free acidity was not as rapid as that following mucin or its derivatives and furthermore, no secondary hyperstimulation appeared.

Contrasting these results with the ones obtained following the mucin products reveals interesting data, to wit: (1) Gastric mucin established an achlorhydria for one-half hour in the three patients studied (4B, 6M, 7O). The same obtained after milk and cream in three cases, but in the fourth was prolonged to three-fourths of an hour. Further, the acid combining power of milk and cream was more sustained than that of gastric mucin. (2) The achlorhydria following "pure" mucin (4B, 8B) may be considered

TABLE VII

COMPARATIVE EFFECTS OF GASTRIC MUCIN, PEPTONE AND MILK AND CREAM UPON GASTRIC ACIDITY

(PATIENT 7O)

TEST MEAL		¼ HR.	½ HR.	¾ HR.	1 HR.	1¼ HR.	1½ HR.	1¾ HR.	2 HR.
Oatmeal gruel	FA	0.0	10.0	34.0	45.0	50.0	46.0	44.0	---
(Control)	TA	19.0	29.0	66.0	74.0	67.5	68.0	65.0	
Oatmeal gruel	FA	0.0	0.0	23.5	55.0	51.0	58.5	56.0	59.0
+ ½ oz. gastric mucin	TA	35.5	51.0	83.5	104.0	92.0	92.0	98.0	97.0
Oatmeal gruel	FA	0.0	0.0	0.0	15.0	35.0	42.0	30.0	34.0
+ ½ oz. peptone	TA	50.0	58.0	64.5	88.0	95.0	90.0	80.0	76.0
Oatmeal gruel	FA	0.0	0.0	13.0	26.0	31.0	34.5	26.0	21.0
+ 3 oz. milk and cream	TA	21.0	45.0	46.5	55.0	64.0	73.5	57.0	54.0

TABLE VIII

COMPARATIVE EFFECT OF PURE MUCIN AND MILK AND CREAM UPON GASTRIC ACIDITY

(PATIENT 8B)

TEST MEAL		¼ HR.	½ HR.	¾ HR.	1 HR.	1¼ HR.	1½ HR.	1¾ HR.	2 HR.	2¼ HR.	2½ HR.
Oatmeal gruel	FA	3.0	30.0	36.0	51.0	58.3	55.0	---	63.0	---	---
(Control)	TA	27.0	53.0	63.0	70.0	75.0	72.0		93.0		
Oatmeal gruel	FA	0.0	0.0	25.0	24.0	36.0	51.0	56.0	71.5	85.5	74.0
+ ½ oz. pure mucin	TA	43.0	60.0	76.0	82.5	92.0	102.0	107.0	105.5	104.4	99.0
Oatmeal gruel	FA	0.0	0.0	10.0	31.0	33.0	39.5	---	---	---	---
+ 3 oz. milk and cream	TA	19.0	32.0	48.0	75.0	70.5	77.5				

as of approximately the same duration as milk and cream, but again, the neutralizing effect was more prolonged with the latter. (3) Peptone effected a more extended absence of free HCl than did milk and cream (three-fourths to one hour compared with one-half to three-fourths hour) in the three cases studied (4B, 6M, 7O), although in two of these, the neutralizing action was less sustained than when using milk and cream. In three of four patients (4B, 6M, 8B) mucin or its derivatives was productive of a hyperstimulation, an end-result not found with milk and cream.

DISCUSSION

In our studies on patients with chronic peptic ulcer, the achlorhydria following one-half ounce of gastric mucin, persisted for one-fourth to one and one-half hours in thirteen experiments, not exceeding one-half hour in the majority, although the total average was only forty minutes (Table IX). Giving one ounce of gastric mucin prolonged the period of achlorhydria to one hour in patient 4B, doubling the effect obtained with the smaller dose. With one-half ounce of gastric mucin, the free acidity was less than that of the controls for a total period of one-half to one and one-half hours, in the majority being one-half to three-fourths of an hour, including the period of absence of free HCl. That the neutralizing substances constituting 40 per cent of mucin play an important rôle in its antacid properties has been adequately demonstrated, since one-half ounce of the peptone-ash fraction effected an achlorhydria for one-half to one hour in three instances. In Fogelson's experiments on Pavlov pouch dogs, achlorhydric periods as long as five to seven hours were observed when one-half ounce of gastric mucin together with one pound of meat was given. This disparity between our results and those of Fogelson may be dependent upon the prolonged buffering action of the meat.

Our studies on patients further corroborate the secretagogue action noted by Fogelson[11] on dogs and confirmed by Ivy and Kim,[14] for, of 21 experiments with mucin and its derivatives, a secondary hyperstimulation of a mild or moderate degree was present in 14, an incidence of 66 per cent (Table IX). Since the completion of our work, Rivers, Vanzant and Essex[15, 16] reported the finding, in some of their patients, of a marked elevation in the acidity in association with an increased gastric secretion, the same being observed in dogs subsequent to the feeding of a regular meal containing 10 gm. of mucin. The resulting curves simulated those following histamine stimulation. Three of four lots of mucin gave all the biologic tests for histamine, but none was detected in the fourth.* More recently, Brown, Cromer, Jenkinson and Gilbert[17] reported "no marked secretagogue effect" from their mucin preparation.

The comparative results following the milk and cream mixture are particularly enlightening, despite the paucity of patients studied. Free acid was absent for one-half to three-fourths of an hour after the administration of the milk and cream, comparing favorably with the average response to one-half ounce of gastric mucin. The free acidity was less than that of the controls (at corresponding intervals) for one to one and three-fourths hours including

*The mucin used by Rivers, Vanzant and Essex was of a different source from that used by us. The presence of histamine in mucin remains an open question.

TABLE IX

A SUMMARY OF THE RESULTS OBTAINED IN THE STUDY OF EIGHT PATIENTS

	TOTAL NO. EXPER.	FREE HCl PRESENT THROUGHOUT EXPER. IN	FREE HCl ABSENT FOR TOTAL PERIOD OF					FREE HCl TITER LESS THAN CONTROL (AT CORRESPONDING INTERVAL) FOR TOTAL OF						SECONDARY HYPERSECRETION
			¼ HR.	½ HR.	¾ HR.	1 HR.	1¼ HR. 1½ HR.	0	¼ HR.	½ HR.	¾ HR.	1 HR.	1¼ HR. 1½ HR. 1¾ HR.	
Controls	14	7	5	2										
Gastric mucin ½ oz.	13	0	2	7	1	1	2	1	1	3	5		3	8
Gastric mucin 1 oz.	1	0				1						1		1
"Pure" mucin ½ oz.	3	0	1	2							2	1		2
"Pure" mucin 1 oz.	1	0		1								1		1
Peptone ½ oz.	3	0		1	1	1					1	1	1	2
Milk and cream 3 oz. (equal parts)	4	0		3	1					1	1	1	1	0

the period of achlorhydria, thereby showing a more prolonged neutralizing effect than one-half ounce of gastric mucin. Moreover, in no case did the milk mixture produce a hyperstimulation (Table IX). Such findings lead us to conclude that the greater virtue of gastric mucin when and if ultimately proved, must rest upon some factor other than its antacid property, such as a demulcent action, functioning as a protective coating to the ulcer, or upon some hitherto unknown or unsuspected factor.

CONCLUSIONS

1. The acid combining power of gastric mucin varies in the same or different individuals. The average period of achlorhydria produced by one-half ounce of commercial mucin lasts forty minutes; the maximum observed is one and one-half hours.

2. The peptone ash fraction plays a considerable rôle in the antacid property of commercial mucin (gastric mucin).

3. The acid combining power of one-half ounce of gastric mucin is greater than that of a bland feeding.

4. In the majority of experiments in which gastric mucin or its derivatives were tested, a mild or moderate secretagogue action is demonstrable.

5. One-half ounce of gastric mucin produces an average achlorhydric period which compares favorably with that of a three ounce mixture of milk and cream but has the disadvantage of a secretagogue action.

6. The possible virtue of gastric mucin in peptic ulcer therapy is dependent upon some factor other than its antacid property, such as a demulcent action.

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LABORATORY METHODS

A MODIFIED METHOD FOR DETERMINING ORGANIC PHOSPHORUS*

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THERE has been much controversy concerning the probable presence of a protein other than bioplasm stored for the purpose of catabolism which has been designated as "reserve" or "deposit." Cahn and Bonat, in 1927, stated that the phosphorus contents of the isolated proteins of the liver of emaciated dogs, guinea pigs, and rabbits were greater than those of the corresponding proteins of respective obese animals. These results were interpreted as indicating the presence of reserve protein. In attempting to duplicate this work, I developed a modified method for determining organic phosphorus in small quantities of protein which I recommend as being quite satisfactory, and with which this paper is concerned.

The determination of organic phosphorus consists of: (1) ashing the organic material; (2) converting the phosphorus to a definite chemical state; (3) getting the phosphorus into solution, and (4) applying a quantitative method.

The principal methods for ashing organic material are the wet and the dry. Neumann, Hartwell, Bosworth, and Kellogg, and Meigs, Blatherwick, and Cary used equal parts of concentrated nitric and sulphuric acids for ashing protein. Bauman stated that Raper, Taylor and Miller, Hartwell, Bosworth, and Kellogg preferred the dry ashing method. Bauman demonstrated errors in organic phosphorus determinations resulting from losses by volatilization and failure to convert all phosphorus to orthophosphoric acid. Hillebrand and Lundell demonstrated that all phosphorus could be safely converted to orthophosphoric acid only if heated in excess of concentrated sulphuric acid and that no volatilization would occur at a temperature of 150° C. or below. Schulz ashed feces by means of digestion with concentrated sulphuric acid, and then added concentrated nitric acid drop by drop.

For the determination of phosphorus in solution, Neumann, in 1902, introduced the titrimetric method which Taylor and Miller, in 1914, improved.

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Gibson and Estes, in 1909, introduced the uranium acetate method; Raper, in 1914, described a gravimetric method using lead phosphomolybdate as the end product; King, in 1918, used $Mg_2P_2O_7$ as the end point; Jones and Perkins, in 1923, used $MgNH_4PO_4$ as the end point and dried the product to constant weight by the use of a vacuum at room temperature. Bloor, in 1915, introduced the nephelometric method; Greenwald, in 1915, introduced the first colorimetric method, the end point of which was really a yellow suspension rather than a true color. Bell and Doisy, in 1920, introduced a method which in principle depended on the reduction of phosphomolybdic acid by hydroquinone in an alkaline medium. Briggs, in 1922, and again in 1924, offered modifications. Fiske and Subbarow, in 1925, demonstrated the errors of all existing colorimetric methods and offered a procedure which consisted of the reaction of orthophosphoric acid, ammonium molybdate, sodium sulphite, and 1-2-4 aminonaphthol sulphonic acid in 0.5 N sulphuric acid medium. Only five minutes were required for the development of the color, which was quite stable. Berggren has recently applied this method for determining phosphorus in casein.

PROCEDURE

After many attempts to determine phosphorus quantitatively in small samples of isolated protein, without obtaining satisfactory checks, the following procedure was finally adopted:

The ammonium molybdate used in the colorimetric method of Fiske and Subbarow was dissolved in water and the acid required to make the final volume 0.5 N was calculated and utilized in the ashing process. A quantity of powdered protein containing from 0.2 to 0.6 mg. of phosphorus was carefully weighed and washed with double distilled water into a hard glass test tube. One and four-tenths cubic centimeters of concentrated sulphuric acid was added and the tube was placed overnight in an electric oven kept at 100° C., during which time digestion occurred and all the water evaporated. The next morning the tube was placed in a support, a few drops of water were added, and a method was arranged to keep the mouth of the tube cooled. This was done by making a U in the center of a glass tube, blowing a bulb at the bend of the U which would occlude about two-thirds of the mouth of the test tube, and attaching a spicule of glass at the most dependent portion of the bulb which extended slantingly to the side of the test tube (Fig. 1). Through this apparatus cool water flowed continually. The solution in the test tube was heated by means of a microburner until steam appeared and the flame was adjusted to keep the temperature constant. Then concentrated nitric acid was added drop by drop; the reaction resulting from one drop was allowed to subside before the next drop was added. When the solution had become yellow, a small quantity of aluminum sulphate was added which acted as a catalyst, and the procedure was continued until the solution was colorless. Then the contents were washed into a 100 c.c. volumetric flask and the colorimetric method of Fiske and Subbarow was carried out, using the 2.5 per cent ammonium molybdate solution made up with water rather than with the 5 N sulphuric acid except in the case of the standard.

COMMENT

Digestion of the protein by sulphuric acid may be effected by water-bath. The few drops of water added to the digested solution served as a thermometer during oxidation. The appearance of white fumes would indicate that the temperature was approximately 150° C. and the flame should be lowered. A catalyst was necessary to destroy at a low temperature the color formed

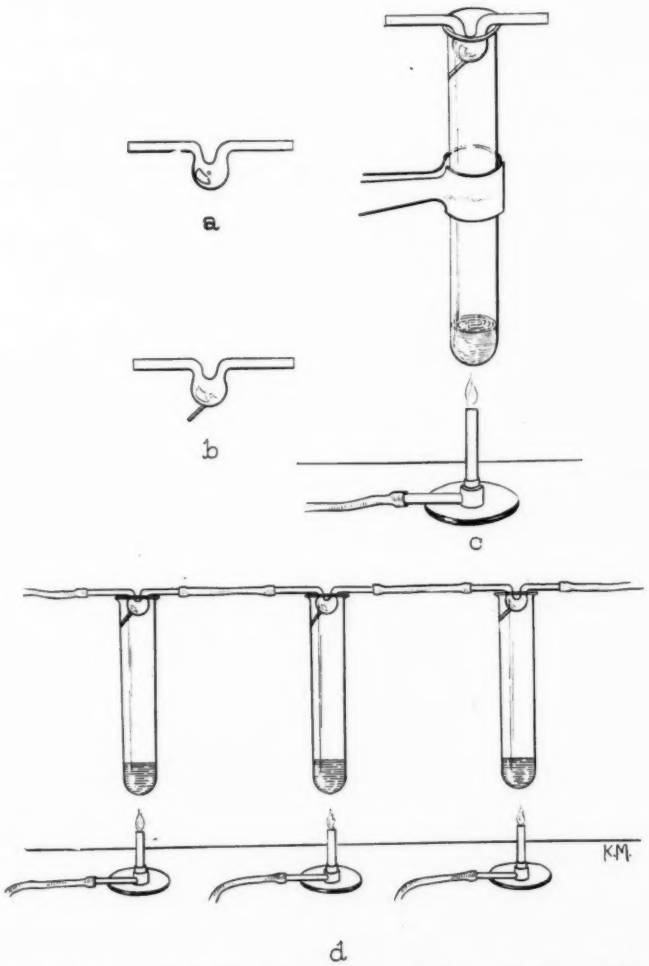

Fig. 1.—*a*, U tube converted into a cooling apparatus; *b*, cooling bulb equipped with a glass spicule; *c*, a single apparatus for digesting organic material, and *d*, three specimens being digested simultaneously.

by a reaction between the benzene ring of the protein and the nitric acid. Mercuric sulphate was the best catalyst but it interfered with the colorimetric method used. A mark should be placed on the test tube to indicate 1.4 c.c. so that the concentrated sulphuric acid used during the oxidation can be replaced before applying the colorimetric test. The spicule of glass on the bulb was necessary to lead the condensed fluid gently back, thus avoiding an explosive

reaction which would result in the loss of some of the solution. Dozens of specimens can be digested overnight and about three specimens can be oxidized simultaneously.

The accuracy of the method of ashing was based on obtaining checks in duplicate determinations on the same powdered protein, and on recovering a known amount of phosphorus that had been added to the protein. The average result of four phosphorus determinations was used as the amount of phosphorus in the protein to which a known quantity was added.

RESULTS

LIVER PROTEIN	PHOSPHORUS, PER CENT	AVERAGE, PER CENT
Sample 1	0.212	0.212
	0.212	
	0.209	
	0.213	
Sample 2	0.360	0.365
	0.369	
	0.370	
	0.363	

Five and two cubic centimeters of standard phosphate solution were added to known weights of liver protein, sample 1, containing 0.212 per cent phosphorus.

PHOSPHORUS ADDED, MG.	PHOSPHORUS RECOVERED, MG.	AVERAGE MG.
0.4	0.404	0.399
	0.397	
	0.395	
	0.400	
0.16	0.161	0.158
	0.157	
	0.159	
	0.157	

CONCLUSION

This method for determining phosphorus fulfills the requisites of Bauman to prevent losses by volatilization, and the conditions of Hillebrand and Lundell for complete conversion of all phosphorus to orthophosphoric acid. It is rapid, especially adapted to the use of small samples of organic material, and has an accuracy of $\pm$ 2 per cent.

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SEPARATION AND DETERMINATION OF BISMUTH AND ARSENIC IN BIOLOGIC MATERIAL*

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DURING an investigation of the excretion of arsenic by patients who had received bismuth previously, or who were receiving both arsenic and bismuth simultaneously or intermittently, the problem arose whether the amount of each of these elements could be determined in the same specimen of blood.

METHOD

Hanzlik, Mehrtens[1] and coworkers studied the excretion of bismuth in patients who received bismuth alone; analytical complications from arsenic were thus absent.

In the experiments here reported hydrazine sulphate was adopted as the most suitable reagent for the reduction of pentavalent arsenic. Since Jannasch and Seidel[2] introduced hydrazine sulphate for the separation of arsenic from other metals in the analysis of minerals, many other investigators[3, 4, 5, 6] have used hydrazine sulphate for their quantitative microanalysis of arsenic.

The use of hydrazine sulphate not only permits the determination of bismuth in the residue from the arsenic distillation but at the same time replaces with great advantage the less stable $FeCl_2$ (see Maechling and Flinn[7]).

With adequate modifications the distillate can be used for the colorimetric or volumetric[8] determination of arsenic.

PROCEDURE

The following procedure was adopted for the separation and determination of arsenic and bismuth in blood: 5 c.c. of blood mixed with known amounts of arsenic and bismuth were oxidized by means of 30 c.c. of a mixture of nitric and sulphuric acids (9:1), first in casseroles covered with watch glasses on the water-bath and then in Kjeldahl flasks with the addition of 30 c.c. of concentrated sulphuric acid. The colorless acid digest was diluted with 70 c.c. of water and boiled to destroy traces of nitrosyl sulphuric acid; the Kjeldahl flask with the digest was connected to an Allihn form of condenser, a safety bulb being placed between the condenser and the receiving cylinder.

A mixture of 10 gm. of NaCl, 1 gm. of NaBr and 1 gm. of hydrazine sulphate was added at once to the acid digest in the Kjeldahl flask and the

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TABLE I

ARSENIC AND BISMUTH RECOVERIES FROM BLOOD

ARSENIC ADDED	TOTAL ARSENIC	ARSENIC AFTER DEDUCTION OF BLANK	BISMUTH ADDED	BISMUTH FOUND
GAMMA	GAMMA	GAMMA	MG.	MG.
None	4.4	None	None	None
None	4.0	None	None	None
None	4.5	None	None	None
10.0	14.0	9.7	None	None
10.0	14.2	9.9	None	None
10.0	14.4	10.1	None	None
None	4.6	None	1.0	1.0
None	4.3	None	1.0	1.08
None	4.6	None	1.0	1.16
10.0	14.3	10.0	1.0	1.0
10.0	13.9	9.7	1.0	0.97
10.0	14.8	10.6	1.0	1.1
10.0	14.4	10.1	1.0	1.0
10.0	13.9	9.7	1.0	0.97

*In the determinations marked with an asterisk in the tables 15 gm. of NaCl and 1.5 gm. NaBr and 1.5 gm. hydrazine sulphate were used; in all other instances: 10 gm. of NaCl, 1.0 gm. of NaBr and 1.0 gm. of hydrazine sulphate were used for the reduction and distillation of arsenic.

TABLE II

ARSENIC AND BISMUTH RECOVERIES FROM WATER

ARSENIC ADDED	TOTAL ARSENIC	ARSENIC AFTER DEDUCTION OF BLANK	BISMUTH ADDED	BISMUTH FOUND
GAMMA	GAMMA	GAMMA	MG.	MG.
None	4.0	None	1.0	1.1
None	3.9	None	1.0	0.9
None	4.2	None	1.0	0.9
None	3.9	None	1.0	1.0
None	5.7*	None	2.0	1.9
None	5.5*	None	2.0	2.3
20	25.1*	19.6	2.0	2.1
20	23.9	19.9	0.4	0.4
25	29.1	25.1	0.5	0.5
25	29.1	25.1	1.0	0.9

arsenic distilled off in the usual way, starting with a very low flame and passing air at the slowest possible rate.

The arsenic trichloride distilled over and was caught and oxidized in the receiving cylinder containing 10 c.c. of concentrated nitric acid and 5 c.c. of bromine water. The resulting solution was evaporated to dryness on the water-bath and the pentavalent arsenic determined colorimetrically.

To the residue in the Kjeldahl flask containing the bismuth, 20 c.c. of concentrated nitric acid were added in order to decompose the excess of hydrazine sulphate. The mixture was heated until the appearance of white fumes. The digest was cooled, diluted with water and boiled once more until white fumes reappeared. Bismuth was determined in this solution by Leonard's method. Citric acid was added to the cold solution in every case, because of the presence of iron in blood. The bismuth standard was acidified by means of sulphuric acid in order to obtain similar conditions.

TABLE III

RECOVERIES OF ARSENIC AND BISMUTH IN WATERY SOLUTION

ARSENIC ADDED	TOTAL ARSENIC	ARSENIC AFTER DEDUCTION OF BLANK	BISMUTH ADDEED	BISMUTH FOUND
GAMMA	GAMMA	GAMMA	MG.	MG.
None	5.7*	None	2.0	1.9
None	5.5*	None	2.0	2.3
20	25.1*	19.6	2.0	2.1

TABLE IV

ARSENIC AND BISMUTH RECOVERIES FROM BLOOD IN THE PRESENCE OF ANTIMONY

ARSENIC ADDED	TOTAL ARSENIC	ARSENIC AFTER DEDUCTION OF BLANK	BISMUTH ADDED	BISMUTH FOUND	ANTIMONY TRICHLORIDE ADDED
GAMMA	GAMMA	GAMMA	MG.	MG.	MG.
None	3.8	None	None	None	0.2
None	4.0	None	None	None	0.2
None	4.1	None	0.2	0.2	1.0
None	4.4	None	0.4	0.4	1.0
None	3.8	None	0.4	0.4	0.5
10.0	13.5	9.4	None	None	0.5
10.0	13.7	9.6	None	None	0.5
10.0	13.8	9.7	0.2	0.2	0.1
10.0	13.3	9.2	0.2	0.2	0.1
10.0	14.2	10.1	0.2	0.2	0.1
10.0	14.2	10.1	0.4	0.4	0.5
10.0	14.3	10.2	0.4	0.4	0.5
None	4.2	None	0.4	0.4	0.5

TABLE V

RECOVERIES OF ARSENIC AND BISMUTH FROM WATERY SOLUTION IN PRESENCE OF ANTIMONY

ARSENIC ADDED	TOTAL ARSENIC	ARSENIC AFTER DEDUCTION OF BLANK	BISMUTH ADDED	BISMUTH FOUND	ANTIMONY TRICHLORIDE ADDED
GAMMA	GAMMA	GAMMA	MG.	MG.	MG.
None	3.6	None	None	None	0.5
None	3.6	None	None	None	0.5
10.0	13.5	9.9	None	None	0.5
10.0	13.7	10.1	None	None	0.5
None	3.6	None	None	None	10.0
20.0	23.0	19.4	None	None	20.0

TABLE VI

RECOVERIES OF ARSENIC AND BISMUTH FROM BLOOD IN PRESENCE OF ANTIMONY

ARSENIC ADDED	TOTAL ARSENIC	ARSENIC AFTER DEDUCTION OF BLANK	BISMUTH ADDED	BISMUTH FOUND	ANTIMONY TRICHLORIDE ADDED
GAMMA	GAMMA	GAMMA	MG.	MG.	MG.
None	5.8*	None	None	Faint trace (?)	20.0
None	5.8*	None	None	Faint trace (?)	20.0
20.0	26.1*	20.2	None	Faint trace (?)	20.0
20.0	25.2*	19.3	None	None	20.0

The separation and quantitative determination of bismuth and arsenic were carried out according to the above described method with specimens of human blood, ox blood, and with water containing known amounts of arsenic and bismuth.

TABLE VII

RECOVERIES OF ARSENIC AND BISMUTH FROM WATER IN PRESENCE OF ANTIMONY

ARSENIC ADDED	TOTAL ARSENIC	ARSENIC AFTER DEDUCTION OF BLANK	BISMUTH ADDED	BISMUTH FOUND	ANTIMONY TRICHLORIDE ADDED
GAMMA	GAMMA	GAMMA	MG.	MG.	MG.
None	5.5*	None	None	None	10.0
None	5.3*	None	None	None	10.0
20.0	25.1*	19.7	2.0	2.1	20.0
20.0	26.0*	20.1	2.0	2.8	20.0
None	5.9*	None	None	Faint trace (?)	20.0

It seemed advisable also to study the influence of the presence of antimony upon the recoveries of arsenic and bismuth. Antimony trichloride was added to some of the blood specimens and also to the watery solutions of arsenic and bismuth compounds in order to investigate the influence of antimony upon the separation and determination of arsenic and bismuth.

SUMMARY

1. The method suggested in this paper permits the separation and quantitative determination of both bismuth and arsenic in the same biologic specimen (blood).

2. The presence of antimony in reasonable amounts does not interfere with the determination of arsenic in the distillation products, nor with the determination of bismuth in the residue according to Leonard's method.

3. The amounts of salts resulting from the distillation process is not large enough to interfere with the determination of bismuth in the residue after the volatilization of the arsenic trichloride.

4. It is necessary to carry out blank determinations simultaneously with every new batch of reagents used, since the amounts of arsenic present in the reagents varies.

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A CLINICAL MODEL OF THE HADEN-HAUSSER HEMOGLOBINOMETER*

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IN 1930[1] I described a new hemoglobinometer with which hemoglobin estimations can be made simply and accurately. This instrument has proved most satisfactory but its distribution is necessarily limited by reason of its initial cost. It is the hemoglobinometer of choice if available. Recently a much less expensive hemoglobinometer has been devised, which utilizes the principle employed in the larger instrument, and is sufficiently accurate for routine clinical work.

Description.—The principal components of the instrument are illustrated in Fig. 1, the parts of the capillary comparison cell in Fig. 2, and the instrument assembled for use in Fig. 3.

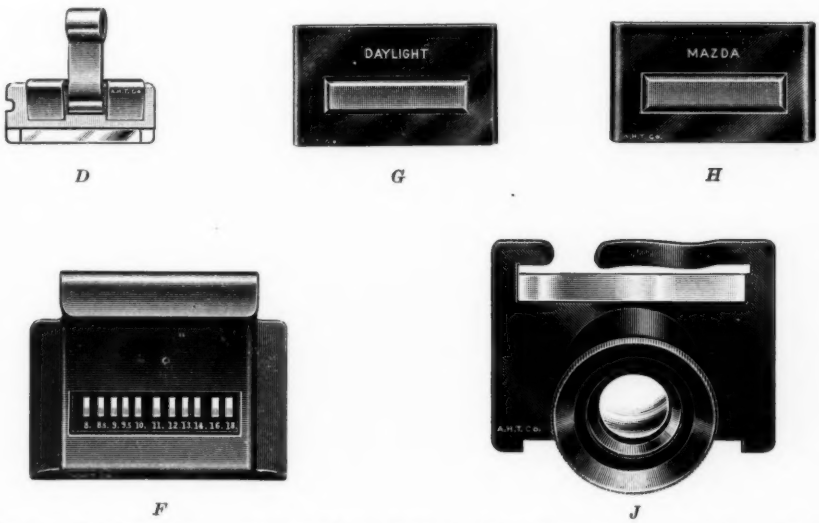
A wedge-shaped chamber to hold the diluted blood is made in the capillary comparison cell, *D* (Fig. 1) by the combination of glass wedge, *A* (Fig. 2) and plane glass plate, *C* (Fig. 2), if the hemoglobin is within the 8 to 18 gram range or by glass wedges *A* and *B*, which doubles the depth of the solution, if the hemoglobin is below 8 grams per hundred cubic centimeters. The other essential parts of the instrument are a comparator with a series of rectangles, the lower half of which is the color standard complement and the upper half of which is the acid hematin complement when the filled comparison cell is in place for reading. The cell *D*, and the comparator *F*, together take the place of the comparison slide used in the laboratory model of the Haden-Hausser hemoglobinometer. A magnifier with a track on which the comparator slides, is added to facilitate reading. Light filters for both daylight (Fig. 1, *G*) and artificial light (Mazda bulb) (Fig. 1, *H*) complete the instrument.

Method of Use.—The blood is diluted 1:10 with tenth-normal hydrochloric acid in a Thoma white cell pipette and the capillary emptied immediately. After standing for thirty minutes, the wedge-shaped chamber of the comparison cell is filled, and the cell placed in the sliding comparator. The sliding comparator carrying the comparison cell and light filter is now suspended on the slide track of the magnifier. On viewing the comparator slide through the magnifier several rectangles are seen. The lower color standard complement is the same throughout as the glass is of uniform thickness; the acid hematin complement is different in each rectangle since the diluted blood is in a wedge-shaped chamber. The comparator is then moved from right to left across the field of view until a close match is found between the upper or acid hematin complement and the lower or color standard complement of one of the illumi-

*From the Cleveland Clinic.

This instrument was developed and is now being made by C. A. Hausser and Sons and is sold by A. H. Thomas Company, West Washington Square, Philadelphia.

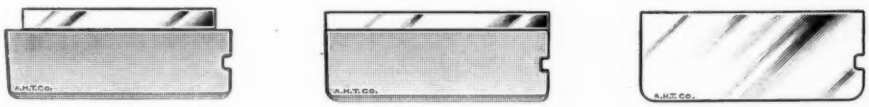
nated rectangles. This rectangle is next brought into the center of the field. The upper half of the rectangle to the left is then a darker tint and of that to the right a lighter tint, if the match is correctly made.



D *G* *H*

F *J*

Fig. 1.—Principal components of the hemoglobinometer. *D*. Capillary comparison cell held in spring clip. *F*. Sliding comparator with hemoglobin-gram scale. This carries both the comparison scale and the light filter. *G*. Light filter for use with daylight. *H*. Light filter for use with Mazda lamp. *J*. Magnifier with support and slide track to carry comparator *F*.



A *B* *C*

Fig. 2.—Components of capillary comparison cell. *A*, glass wedge; *B*, glass wedge; *C*, plane glass plate.

A *B*

Fig. 3.—Hemoglobinometer assembled for use. *A*, front view; *B*, rear view.

The numerical value in grams of hemoglobin per hundred cubic centimeters of blood is indicated beneath the matched rectangle. If the hemoglobin is too low to read, a capillary cell made with wedges *A* and *B* instead

of *A* and *C* is filled with the diluted blood remaining in the pipette. The reading is then made and the value divided by 2 to obtain the hemoglobin in grams per hundred cubic centimeters.

Experimental Observations.—In calibrating this instrument, I have made comparative readings with the oxygen capacity method of Van Slyke, the iron content method of Wong, and with values obtained with the laboratory model of the Haden-Hausser hemoglobinometer. These results are shown in Table I. It is apparent that readings well within the needs of clinical work can be made with this instrument. The readings can be made at the bedside, if desired. The cost of the instrument is small and the standard is permanent.

TABLE I

COMPARISON OF HEMOGLOBIN READINGS WITH CLINICAL MODEL OF THE HADEN-HAUSSER HEMOGLOBINOMETER AND OTHER METHODS

SPECIMEN NO.	CLINICAL MODEL HADEN-HAUSSER HEMOGLOBIN-OMETER	LABORATORY MODEL HADEN-HAUSSER HEMOGLOBIN-OMETER	OXYGEN CAPACITY METHOD VAN SLYKE	IRON CONTENT METHOD NEW WONG
	Gm. per 100 c.c.	Gm.	Gm.	Gm.
1.	8.0	8.0	7.9	7.7
2.	9.0	9.0	8.6	8.5
3.	10.0	10.0	9.7	9.5
4.	11.5	11.5	11.2	10.9
5.	13.0	13.0	13.0	12.8
6.	14.0	14.0	14.3	14.0
7.	15.0	15.5	15.7	15.4
Mean	11.5	11.6	11.5	11.3

Discussion.—The instrument is calibrated in grams only, using the two standard methods of calibration; namely, the oxygen capacity and iron content methods. The readings obtained with the hemoglobinometer can be compared then with those obtained by the two chemical methods. This is the first requisite in all hemoglobin estimations. The second problem concerns the translation of values so obtained into percentage terms. I have discussed the question of hemoglobin standards recently elsewhere[2] and have offered a

TABLE II

SPECIMEN NO.	RED CELL COUNT IN MILLIONS PER CM.	HEMOGLOBIN IN GRAMS PER 100 C.C. WITH CLINICAL MODEL, HADEN-HAUSSER HEMOGLOBINOMETER
	Millions	Grams
1.	5.02	16.0
2.	4.00	12.5
3.	4.65	15.0
4.	5.50	17.0
5.	4.78	15.0
6.	4.57	14.0
7.	4.25	13.5
8.	5.31	16.0
9.	5.80	17.0
10.	4.92	15.0
Mean	4.88	15.1

simple solution of the confusing problem of choosing between standards suggested by different workers in this field. It is a very easy matter for each laboratory worker using the hemoglobinometer to determine for himself the standard to use. The most important point is the use of a standard which in the hands of the worker employing it gives a color index of 1.00 ± 0.10. This is done by making hemoglobin determinations and accurate red cell counts simultaneously on each of about ten normal bloods. The number of grams of hemoglobin per hundred cubic centimeters per five million cells, or the hemoglobin coefficient, is calculated from these data, and the figure so obtained taken as 100 per cent in figuring results in percentage terms. This is illustrated in Table II.

Since the average normal blood in the example given with a red cell count of 4,880,000 per cubic centimeter contains 15.1 grams of hemoglobin per hundred cubic centimeters, it is apparent that the hemoglobin content would be 15.4 grams if the count were 5,000,000 red cells. The figure so obtained (15.4 grams) is thus taken as 100 per cent. When so determined, the color index in normals should always be 1.00 ± 0.10. In reporting results in percentage obtained in this manner, confusion is prevented by indicating that 100 per cent equals the hemoglobin in grams per hundred cubic centimeters per five million cells in normal persons. The actual number of grams per hundred cubic centimeters makes no difference if the color index in normal persons is 1.00 within the limits of error.

Summary.—A new inexpensive hemoglobinometer is described which employs: (1) the acid hematin principle of hemoglobin determination; (2) a permanent standard; (3) a very low dilution ratio (1 to 10) in a standard white cell pipette; (4) reads directly in grams; and (5) is calibrated by the oxygen capacity and iron content methods.

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A NOTE ON THE PRESERVATION OF ACID-FAST BACTERIA IN VACUO*

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IT HAS been the usual practice in this laboratory to maintain the stock of mycobacteria by subculture every four to six weeks. This procedure, although effective, has the following disadvantages: (1) if, as may occasionally happen, too long a period is allowed to elapse between transfers, i.e., eight weeks or longer, some strains may have died out and been lost; (2) considerable time is used for this purely routine work; (3) the necessary media are relatively expensive and troublesome to prepare.

It was thought that the method of Brown for preserving the common pathogens and saprophytes in vacuo might be adapted to mycobacteria and afford a simple means of maintenance for long periods without transfer. Brown's method in brief is as follows: a number of small pieces of filter paper about ¼ by ½ inch are placed in small glass vials (1½ inches by ⅜ inch) plugged with cotton and sterilized. Using a vial for each strain a loopful of a young culture is placed on each bit of paper in the vial. A number of vials with various strains of bacteria are placed in half-pint or pint bottles over calcium chloride. A small manometer is put within the bottle so it can be read from outside. A little vaseline is placed around the lip of the bottle and a special metal cap is lightly placed on top. The bottle is then evacuated under a bell jar with the use of a good vacuum pump for fifteen or twenty minutes until a high vacuum is obtained. Air is allowed to enter the bell jar suddenly which forces the cap down tightly on the bottle. The bottle is then placed in a hand crimping machine and the cap crimped. It is then stored in the ice box. (The bottle caps and crimping machine may be obtained from the Crown, Cork and Seal Company of Baltimore.)

To preserve acid-fasts the above procedure was modified slightly. The vials were plugged and sterilized empty. With a sterile platinum spatula kernels of the hard wrinkled growth were transferred to the vials, the vials placed in a bottle, and the bottle evacuated and crimped as above described and left in the refrigerator for eleven months. Four-week-old cultures from Petroff and Lubenau medium slants were used. Thirty-one strains, each strain in one vial, were stored in this way. They are shown in Table I.

After eleven months the bottle was opened and the hard dry "kernels" were easily broken up with a spatula and a little was transferred to slants of Lubenau, Petroff, Sweany cream medium and also Petragnani medium in the case of pathogens and glycerin agar slants in the case of the saprophytes.

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TABLE I

MYCOBACTERIUM TUBERCULOSIS

HUMAN	BOVINE	AVIAN	SAPROPHYTES
Saranac H 37	B 1	BA1 Parrot	B. E. S.
Lov. 7	Princeton 32	BA1 Pheasant	Timothy 6292
Lov. 9	Saranac B 1	BA1 Swine	Saranac Timothy
Lov. 62	Park 643	Van Es H3701	Timothy A.M. 213
Lov. 71	Zinsser B 1	Van Es stock	Butter R.
	Opie B 1	Van Es 4957	B. moelleri
	Hyg. Lab. 410	Van Es 3732	Red timothy
		Tyzzer A 3	B. smegmatis
			Saranac mist
			Saranac "lepra B"
			B. leprae. Am. Museum "Apa."

In forty-eight hours all of the saprophytes had grown out. In three weeks most of the pathogens showed some growth and in four weeks all of the pathogens showed excellent growth. Of the media used the Petragnai malachite green medium appeared to give the finest growth. Three of the pathogenic strains were evidently contaminated in the vials, for the contaminants as well as the mycobacteria grew out on all cultures. Two of the three contaminated were easily purified by fishing isolated colonies from the Petragnani slants. This was possible because the malachite green dye in the medium inhibited the contaminant while allowing the acid-fast to grow. In the case of the third contaminated strain acid digestion methods had to be resorted to, to purify the culture.

About 1 mg. each of the preserved human strain H 37 and of the bovine strain Princeton 32 was injected into two guinea pigs. The human strain was first injected subcutaneously in the right groin and seven weeks later intraperitoneally. The autopsy was made eleven weeks after the first injection and showed a general glandular enlargement with caseation, a rolled up thickened, caseous omentum, an enlarged granular spleen and implantations on the surface of the liver and the parietes. The bovine strain was inoculated intraperitoneally four weeks before autopsy. The parietes were roughened with innumerable minute translucent tubercles; the spleen was smooth and a little enlarged, the omentum rolled up and caseous, the glands somewhat enlarged and a few very small necroses in the liver. It appears from our results that acid-fast bacteria can be preserved for at least eleven months by the method described without any apparent loss of virulence.

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A METHOD FOR THE COLORIMETRIC DETERMINATION OF THE URINARY CHOLESTEROL*†

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A FEW attempts have been made to determine the amount of cholesterol present in the urine. All methods for the estimation of urinary cholesterol necessitate two steps: the first is complete extraction of the urine with a lipoid solvent, and the second is the determination of the cholesterol content of the extract thus obtained. The extraction procedures generally employed are either of the following: the urine is directly extracted by lipoid solvents or it is first evaporated to dryness and then extracted. The former is a lengthy process requiring repeated extraction and separation of a large sample of urine over a protracted period of time. The latter is cumbersome because the evaporation of a large volume of urine and the complete extraction of the resultant gummy residue is both tedious and difficult. We have been unable to obtain satisfactory extractions of the urine by either of these methods.

Two means for the determination of the cholesterol content of the urinary extract based on the gravimetric and the colorimetric principles have been applied. Gardner and Gainsborough[1] extracted large volumes of alkalinized urine with ether for five to six days. The ethereal extract thus obtained was saponified, and the cholesterol measured by the digitonide method. The extracted urine was further hydrolized with glacial acetic acid. The resultant solution was extracted with ether and the cholesterol content again estimated. They thus found, after acid hydrolysis, what they believed to be an "ethereal sulphate" of cholesterol. The gravimetric method (Windaus[2]) is adequate since the relationship between free cholesterol and its digitonide has been well established. Nevertheless, the difficulties inherent in the performance of a gravimetric determination make this process so complicated that it is not suitable for routine use.

Criticism of the colorimetric method as applied to urine has centered mainly on the pigmentation and occasional turbidity of the extracted solution which renders the final color brownish or yellowish. We have succeeded in obviating this difficulty by the introduction of a procedure which extracts the lipoids while it does not extract the pigments. Furthermore, our method requires only a relatively small amount of urine for each determination; it can be carried out in from two to three hours and is accurate as far as can be judged from estimations of duplicate samples.

The principle of the method is based chiefly on the observation of de Toni[3] who found that the tungstic acid precipitate of blood contains all the choles-

*From the Department of Medicine, New York Post-Graduate Medical School and Hospital. Received for publication, October 21, 1932.
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terol. We have repeatedly confirmed this observation. Gardner and Gainsborough studied the urine from a case of "subacute parenchymatous nephritis"; they acidified the urine with acetic acid and boiled it; the resultant solution was filtered and the precipitate was washed and dissolved in 2 per cent caustic soda; this was then extracted with ether and the cholesterol determined. The filtrate was alkalinized and repeatedly extracted with ether; the thoroughly extracted filtrate was then evaporated to dryness, the residue hydrolized with glacial acetic acid, extracted and saponified. They thus found that 91 per cent of the free and ester cholesterol apart from the "ethereal sulphate" was precipitated along with the proteins. In the method described below all the urinary cholesterol is precipitated with a tungstic acid precipitate. We have found no evidence for the existence of an "ethereal sulphate" of cholesterol.

METHOD

Total Cholesterol.—The urine must be neutral or slightly acid in reaction. If alkaline, it should be neutralized with dilute acetic acid. One hundred cubic centimeters of the urine are measured into a centrifuge bottle of 150 c.c. capacity. To this are added 5 c.c. of a 50 per cent solution of sodium tungstate* and 5 c.c. of a 10/3 N solution of sulphuric acid, the sulphuric acid being added drop by drop. The bottle is then centrifuged for five to ten minutes and the supernatant fluid discarded. About 150 c.c. of boiling distilled water are added to the precipitate which is broken up with a glass rod and subsequently centrifuged. The supernatant fluid is discarded. This is repeated, so that altogether the precipitate is washed two or three times with boiling distilled water. Fifty cubic centimeters of an alcohol ether mixture (3 to 1) are added to the precipitate in the bottle. This is placed on an electric plate and allowed to boil for about one minute. While hot, the extract is filtered through a fritted glass filter of fine porosity into a small clean Erlenmeyer suction flask and slight suction is applied. The precipitate is washed once with 25 c.c. of hot alcohol ether mixture and twice with 15 c.c. of boiling absolute alcohol. The filtrate in the flask is transferred to a beaker and evaporated to dryness over a hot plate. If the original precipitate has been adequately washed with boiling distilled water, the brownish residue will just cover the bottom of the beaker. Fifteen cubic centimeters of petroleum ether (B.P. 30° to 50° C.) are added to the beaker and evaporated to approximately half the original volume on an electric plate. (Avoid open flames.) The petroleum ether extract is decanted carefully into a 20 c.c. volumetric flask. The petroleum ether extraction and the subsequent addition to the flask is repeated two or three times. The combined extract in the 20 c.c. volumetric flask is evaporated to dryness over a steam-bath or in an incubator. To the residue are added 20 c.c. of chloroform. Five cubic centimeters of the chloroform extract are transferred to a test tube. To another test tube are transferred 5 c.c. of a standard solution prepared by dissolving 10 mg. of cholesterol in 100 c.c. of chloroform. The second test tube with the

*The sodium tungstate solution should be approximately neutral in reaction.

standard solution, therefore, contains 0.5 mg. of cholesterol. To each of these are added 2 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulphuric acid. The tubes are placed in a dark closet at room temperature for five minutes, then in a refrigerator for another ten minutes.[4] A comparison is made between the green colors of the standard and unknown solutions. If the color of the unknown deviates by more than 35 per cent to 40 per cent from that of the standard solution a suitable aliquot of the chloroform extract must be used. As an alternative, several standard solutions may be used. A 20 mg. per cent standard, a 10 mg. per cent standard, and a 5 mg. per cent standard may be prepared and the appropriate one employed. We have found the former procedure quite convenient. The amount of total cholesterol in the urine is then calculated by applying the following formula:

$$\frac{R_s}{R_u} \times \text{mg. cholesterol in standard} \times \frac{100}{A} = \text{mg. cholesterol per 100 c.c. of urine}$$

Where R_s = the reading of the standard solution, R_u = the reading of the unknown solution, and A = the amount of urine represented by the chloroform aliquot.

Free and Combined Cholesterol.—After the total urinary cholesterol has been determined, twice the amount of chloroform extract that was necessary for that determination is evaporated to dryness and taken up in about 20 c.c. of an alcohol ether (3 to 1) mixture. The free and combined cholesterol fractions are estimated by fixing the free cholesterol in the form of a digitonide and determining the esters colorimetrically as is done for blood.

The procedure just described is suitable for urines rich in cholesterol as in certain cases of nephritis, and other pathologic conditions. For normal subjects, a larger amount of urine should be extracted; at least 400 to 500 c.c. of urine must be taken for each determination. This is accomplished by combining the petroleum ether extracts from four or five 100 c.c. samples treated as outlined and determining their cholesterol content. The variations in normal and pathologic urines will be discussed in a subsequent paper.

DISCUSSION

The tungstic acid filtrates of urines, from cases often designated as lipoid nephrosis, were evaporated to dryness and extracted very carefully with alcohol ether (3 to 1). Since cholesterol was never found in the filtrates thus studied, it is assumed that all the cholesterol is brought down with the tungstic acid precipitate. However, Gardner and Gainsborough have reported the presence of an "ethereal sulphate" of cholesterol in the filtrate of a deproteinized urine from a case of "sub-acute parenchymatous nephritis." They studied only one sample of urine but we have been unable to confirm this observation in four "nephrotic" urines. The filtrates from such urines were hydrolized with glacial acetic acid by boiling under a reflux condenser for five hours. The resultant solution was evaporated and the residue thoroughly extracted with an alcohol ether mixture. No trace of cholesterol was found in these extracts by the colorimetric method.

The water washes from the precipitate were studied in a manner similar to the urine filtrates, and again no cholesterol was found. Thorough washing

TABLE I

DETERMINATIONS OF THE CHOLESTEROL CONTENT OF NORMAL AND PATHOLOGIC URINES*

URINE	FIRST SAMPLE			SECOND SAMPLE			AVERAGE PER CENT DEVIATION FROM MEAN			REMARKS
	TOTAL CHOL. MG. %	ESTER CHOL. MG. %	FREE CHOL. MG. %	TOTAL CHOL. MG. %	ESTER CHOL. MG. %	FREE CHOL. MG. %	TOTAL CHOL.	ESTER CHOL.	FREE CHOL.	
1	4.52	3.35	1.17	4.57	3.32	1.25	0.6	0.4	3.5	Chronic diffuse glomerular nephritis (with nephrotic component)
2	2.10	1.38	0.72	2.05	1.31	0.74	1.0	2.4	1.5	Chronic diffuse glomerular nephritis
3	2.00	1.23	0.77	1.97	1.08	0.89	0.7	6.5	7.4	Chronic diffuse glomerular nephritis
4	2.04	0.96	1.08	2.16	1.08	1.08	2.8	5.8	0.	Chronic diffuse glomerular nephritis
5	3.00	1.41	1.59	3.03	1.53	1.5	0.5	3.9	3.2	Chronic diffuse glomerular nephritis (with nephrotic component)
6	0.04			0.04			0.			Normal
7	4.47			4.69			2.3			Chronic diffuse glomerular nephritis (with nephrotic component)
8	0.31			0.29			4.0			Normal
9	1.19			1.19			0.			Diabetes mellitus
10	0.88			0.87			0.4			Obstructive jaundice
Average							1.2	3.8	3.1	

*The above figures are no indication of the total urinary cholesterol since the urine volume output is not considered.

of the tungstic acid precipitate allows for better extraction with the alcohol ether mixture. For example, four 100 c.c. samples of urine from a case of "lipoid nephrosis" were precipitated with tungstic acid. In two of these, the precipitate was thoroughly washed with boiling distilled water; the other two were not washed. The extract from the washed precipitates contained 4.47 mg. per cent and 4.69 mg. per cent of total cholesterol respectively. Those from the unwashed precipitates contained 2.75 mg. per cent and 3.66 mg. per cent respectively.

The extraction of the precipitate twice with the alcohol ether mixture and twice with hot alcohol is adequate. The subsequent extraction of the alcohol ether residue with petroleum ether is also complete as is evidenced by duplicate determinations (Table I).

SUMMARY

A comparatively rapid colorimetric method for the determination of urinary cholesterol in normal and pathologic urines is described.

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3. de Toni, G. M.: The Colorimetric Estimation of Cholesterol and Lecithin in Blood in Connection with Folin-Wu's System of Blood Analysis, J. Biol. Chem. **70**: 207, 1926.
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A CONTINUOUS EXTRACTION APPARATUS*†

S. E. Owen, Ph.D., and W. L. Olsen, B.S., Salt Lake City, Utah

ONE phase of our work on pancreatic function tests required the extraction of urine with butyl alcohol. To eliminate considerable time-consuming labor with separatory funnels, the apparatus herein described was devised for the particular purpose of extracting relatively small quantities of urine. We believe that it is simpler in construction than most apparatus, and is more satisfactory for our purpose than any other with which we are familiar.

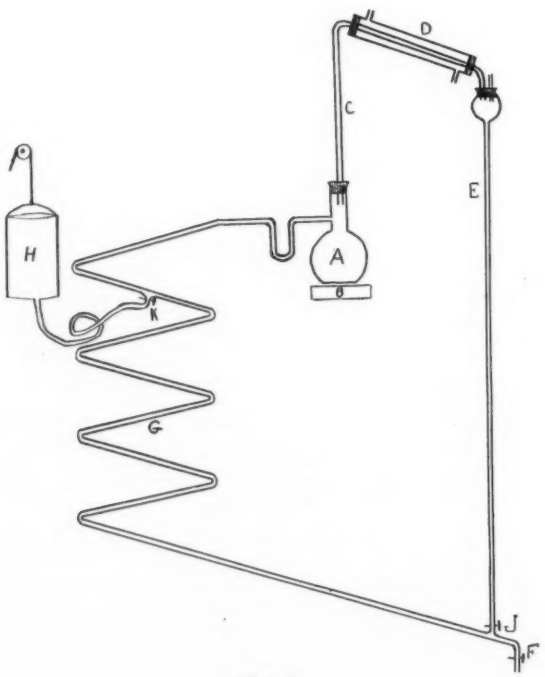

Fig. 1.

The advantages are as follows: (1) It may be used in the extraction of either a stationary column of liquid or a continuously flowing column. (2) Small quantities of a substance may be as conveniently extracted, as larger quantities. (3) Only a small amount of solvent is required. (4) The small bore of the extraction tube renders extraction more thorough and rapid, as do the sharp bends which tend to break the droplets of solvent into smaller droplets. The latter property could be improved by either indenting the tube

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at frequent intervals, or by inserting a fine coiled noncorrosive wire into the extraction tube. (5) By modifying the apparatus to allow a lowering of the distilling flask and a change in the points of entrance of the solvent and the liquid to be extracted, it could be used for extraction with solvents heavier than the liquid to be extracted. (6) The apparatus is simple to construct and the materials required may be found in almost any laboratory.

Construction.—The extraction column *(G)* is made of 8 mm. bore Pyrex or soft glass. This column is given a zigzag shape, the slope being about four inches per foot. While our apparatus rarely had more than four such sections, the only limiting factor to the length of the extraction column would be the room height. The solvent column *(E)* consists of a 4 mm. bore glass tube welded to the larger column. The urine inlet is situated at *(K)* near the top of the zigzag column. Tube *(E)* which carries the butyl alcohol from the condenser must be of sufficient length to allow the butyl alcohol column to overbalance the column of urine and superimposed butyl alcohol in tube *(G)*; after calculating the lengths of the liquid columns required with reference to the specific gravities of the substances employed, it is well to allow one or two inches more on the butyl alcohol column *(E)*. The reservoir for urine *(H)* is placed on an adjustable stand or arranged with a pulley and cord suspension as shown, thus allowing adjustment of the urine level when the apparatus is used for continuous flow extraction.

Use.—In use, the flask *(A)* has placed in it about 100 c.c. of the solvent, and tube *(E)* is filled with the solvent. The material to be extracted is then introduced into the reservoir and the extraction column *(G)* is filled. The stopcock at *(J)* is then opened and extraction commences when the butyl alcohol distills over into the small receiver at the top of tube *(E)*. Heat regulation of the hot-plate *(B)* must be adapted to the individual apparatus; we find that when the returning drops of solvent are spaced about two inches apart, the apparatus functions satisfactorily. When a continuous flow is desired, the stopcock at *(F)* is opened allowing a slow stream of urine to flow out. To prevent excessive refluxing in the vapor column *(C)*, this column is insulated with asbestos rope.

We believe that this apparatus could also be used to advantage in the recovery of drugs from body fluids, the extraction of tissue juices and extractives, and numerous other similar operations.

A SIMPLE METHOD OF REMOVING MUSCULAR TREMOR WAVES FROM THE ELECTROCARDIOGRAM*

C. S. DANZER, M.D., F.A.C.P., BROOKLYN, N. Y.

THE electrocardiogram is admittedly becoming an increasingly indispensable procedure in the diagnosis and prognosis of many heart disorders. It is often sufficiently typical to be diagnostic of several heart conditions.

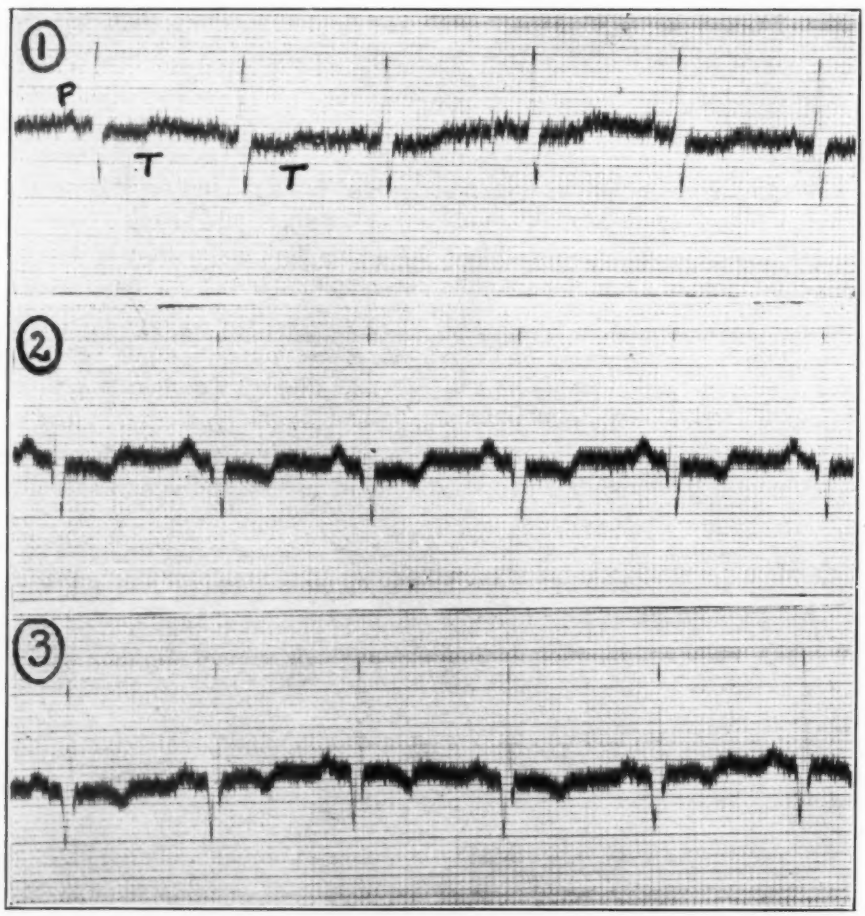

Fig. 1.—Electrocardiogram taken in the usual manner.

Despite adequate care in the taking of the tracing its value is often reduced or lost because the addition of wavelets due to muscular tremors make interpretation difficult.

*From the Cumberland Hospital and Brooklyn Cancer Institute of the City of New York.
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In some cases sufficient rest in the recumbent position before taking the electrocardiogram reduces these, in others this is insufficient. For such cases I have developed a method which is generally successful in removing these wavelets. Its description is the object of the present communication.

The method consists briefly of the administration of 8 c.c. of the elixir of diallyl barbituric acid (Dial) and allowing the patient to lie down for

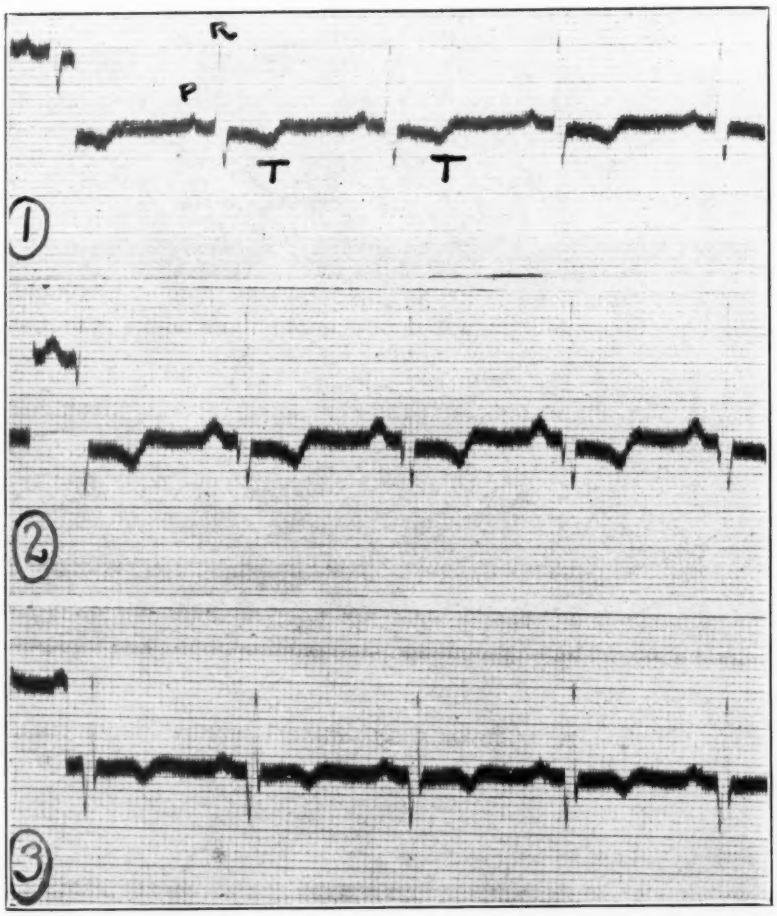

Fig. 2.—Electrocardiogram from same patient after Dial and twenty minutes rest.

twenty or thirty minutes before the electrocardiogram is taken. It is probable that other sedatives may act similarly.

Figs. 1 and 2 were taken before and after the use of the sedative, and are self-explanatory. It may be seen that in Lead 1 the negative T-wave which is such an important finding, has been completely obliterated by the disturbing muscular vibrations.

216 Clinton Avenue.

AN INEXPENSIVE BUT DURABLE METABOLIC CAGE FOR SMALL ANIMALS*

A. RICHARD BLISS, JR., PHM.D., M.D., MEMPHIS, TENN.

THE usual cost of manufacturers' animal cages, which are large enough and strong enough for use in metabolic studies with small animals, such as cats, adult rabbits, and small dogs, is prohibitive for moderate college laboratory budgets, especially in the event a fairly large number of cages is needed. The cage

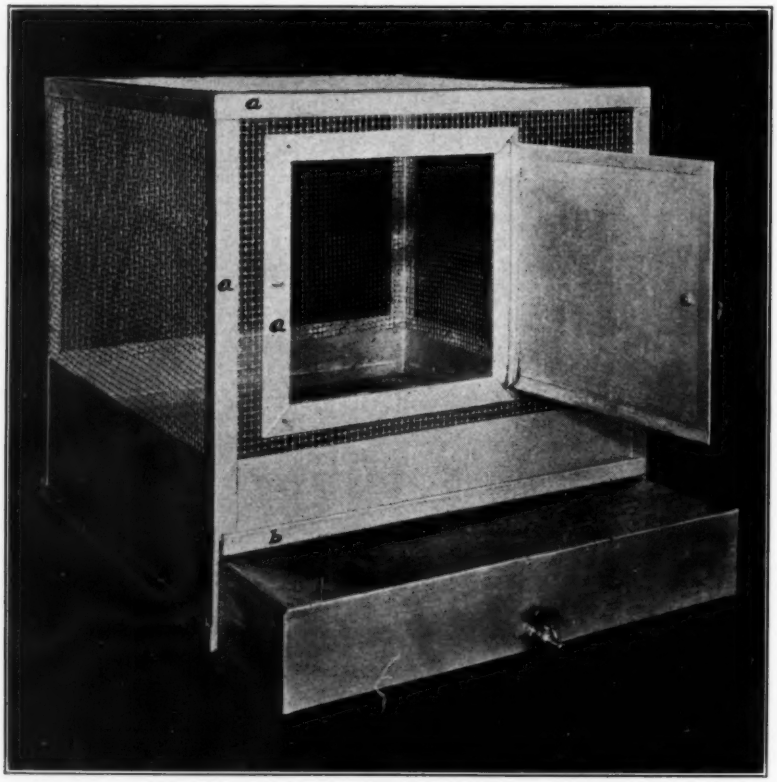

Fig. 1.—An inexpensive, durable cage for small animals made in the University shop.

shown in Fig. 1 was devised in the Division of Pharmacology of the University of Tennessee with the collaboration of Mr. M. B. Parker, B.S., in charge of the "University Apparatus Company," which is housed in the Anatomy Building of this institution.

The cage is 18 inches by 18 inches by 18 inches, and is made of galvanized iron (22 gage) and hardware cloth (4 by 4), the parts soldered together. The edges

*From the Division of Pharmacology, College of Medicine, University of Tennessee.
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(*a*) of the cage are made of bent strips of the galvanized iron (see Fig. 1). The outside depth of the pan is 3½ inches; the bottom of the pan, one piece of galvanized iron, is sloped downward toward the stopcock, the only seam running from the stopcock to the top of the drawer front. The removable, sliding bottom (*b*), supported immediately above the pan, is made of 8 by 8 hardware cloth, edged with galvanized iron, the outer edge bent upward as shown in the illustration. An ordinary stopcock and a hook for the hinged door complete the cage. The total weight is 18 pounds, which permits ready stacking and transfer. The cage is painted inside and out with aluminum paint. The total cost, including materials and mechanician's charge, is $8.50 per cage. Twenty-four of these cages have been in constant use for over a year, and have proved quite satisfactory and durable.

Item

The American Society of Clinical Pathologists

The American Society of Clinical Pathologists held their twelfth annual convention in Milwaukee from June 9 to 12. Dr. A. G. Foord of Pasadena, California, was inducted into office as President for the years 1933 and 1934. The officers elected are as follows:

President-elect: Dr. F. H. Lamb, Davenport, Iowa
Vice President: Dr. J. J. Seelman, Milwaukee, Wisconsin
Secretary-Treasurer: Dr. A. S. Giordano, South Bend, Indiana

The Ward Burdick award was given to Dr. A. H. Sanford of Rochester, Minnesota.

The first prize for Scientific Exhibits was given to a group of four men made up of Drs. A. O. Gettler, H. S. Martland, A. V. St. George, and Charles Norris. Second prize was given to Dr. W. D. Stovall of Madison, Wisconsin.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LEUKOCYTES, Schilling Blood Count in the Pneumonias of Infancy and of Childhood, Rogatz, J. L. Am. J. Dis. Child. **45:** 1022, 1933.

Fifty cases of juvenile, bronchial and lobar pneumonia were studied with reference to changes occurring in the leukocytes during these infections. The Schilling classification of immature cells was used. The daily changes in these elements of the blood were observed and are described in detail. It was possible to anticipate lysis in bronchial pneumonia or the occurrence of the crisis in the lobar types by many hours. Descriptions, with charts and hemograms, are given of striking cases, which illustrate this observation.

From these and other studies it is concluded that the Schilling blood count is of greater value than the ordinary smear in revealing changes in the patient's condition, that it reflects these changes more accurately and somewhat in advance of their clinical appearance and that it should therefore replace the usual differential examination of the blood in routine practice.

LEUKOCYTES, Cytoplasmic Changes in Infection, Sutro, C. J. Arch. Int. Med. **51:** 747, 1933.

The author reports a study of the presence or absence of "toxic granules" in the circulating leukocytes in 200 cases of various infections and determined the "degenerative index" by the formula:

$$\frac{\text{Toxic polymorphonuclear cells}}{\text{Total polymorphonuclear cells}} = \text{index.}$$

1 = 4-plus; ¾ = 3-plus; ½ = 2-plus; ¼ = 1-plus.

In a study of two hundred cases in which the blood was investigated for toxic granules, it was found that the continued presence of a four-plus degenerative index would indicate a serious prognosis. A four-plus degenerative index in cases in which bacteremia or pyemia is suspected points to generalized infection in spite of a negative blood culture. In local infections (mastoiditis, acute appendicitis, etc.) without complications, there will be no toxic granules, or at most the degenerative index will be one- or two-plus. However, should these conditions be complicated by bacteremia or peritonitis, toxic granules appear in many or all of the polymorphonuclear cells.

BILIARY CRYSTALS, Diagnostic Importance of, Rofsky, H. A. Am. J. M. Sc. **185:** 851, 1933.

Various types of biliary crystals found in preoperative specimens of bile were compared with the crystalline elements found in the scrapings of calculi or bile removed from the gall bladder at the time of operation.

In 91.3 per cent of the cases the presence of these crystals in the bile, preoperatively, indicated calculous or noncalculous cholecystitis, irrespective of the history or radiographic findings.

Bile microscopy should be employed more frequently, especially in patients with a more or less persistent gastric history.

BLOOD: Determination of Iron Content of in Children, Sobel, P. I., and Drekter, I. J. Am. J. Dis. Child. **45**: 486, 1933.

Reagents.—Standard Solution A: Dissolve 0.702 gm. of ferrous ammonium sulphate (Merck's reagent) in about 50 c.c. of water, add 20 c.c. of 10 per cent iron-free sulphuric acid, warm and add tenth-normal potassium permanganate solution until a faint pink color persists. Dilute to 1,000 c.c. One cubic centimeter of this solution will contain 0.1 mg. of iron.

Standard Solution B: Dilute standard solution A with an equal volume of water. One cubic centimeter of this solution will contain 0.05 mg. of iron.

Sulphocyanic Acid: Dissolve 100 gm. of ammonium thiocyanate in 100 c.c. of 65 per cent (by weight) sulphuric acid. Measure the volume, transfer to a large separatory funnel and shake the mixture with three fourths of its volume of amyl alcohol. Let the solution settle and discard the aqueous solution. Add an equal volume of water, and shake thoroughly. Preserve the water extract. Repeat the extraction of water, and combine the two extracts, which contain about 7 per cent of sulphocyanic acid. Saturate the water with solid mercuric sulphocyanate so that the excess remains on the bottom of the container. Allow to stand overnight in a dark bottle.

Persulphate Solution: This is a saturated solution of potassium persulphate free from iron in distilled water.

Potassium Chlorate Solution: Dissolve 10 gm. of chemically pure potassium chlorate (iron-free) in 100 c.c. of water.

Extraction Mixture: Combine equal parts of ethylene glycol monobutyl ether and ethyl ether. The ether does not have to be of the highest purity, as the presence of mercuric sulphocyanate prevents the oxidation of sulphocyanic acid by peroxides which may be present in the ether.

Technic: One cubic centimeter of oxalated blood is diluted with 4 c.c. of iron-free distilled water. Using the same pipet with which the blood was measured, transfer 1 c.c. of the diluted blood into a pyrex test tube (2.5 by 20 cm.). And 1 c.c. of concentrated sulphuric acid (free from iron), heat the tube until white fumes are given off, cover it with a small watch glass, and continue heating for two minutes. Remove the flame, place the tube at an angle of 45 degrees, and add 1 c.c. of 10 per cent potassium chlorate carefully, drop by drop. Place the tube in an upright position and heat until white fumes are evolved. Again place the tube at a 45 degree angle; add an additional 0.3 c.c. of the potassium chlorate solution, and boil the mixture until all the chlorine is removed. Allow the tube to cool; dilute the contents with 15 c.c. of water, and add a drop of persulphate solution. Add exactly 25 c.c. of the extraction mixture, followed by 5 c.c. of the sulphocyanic acid reagent. Fit the tube with a rubber stopper and shake thoroughly. The solution separates into two distinct layers, the upper of which is red and the lower colorless. The red color is compared in the colorimeter with a suitable standard solution of iron which has been digested and treated in the same manner as the unknown. Two standards are prepared as described under "Reagents."

Calculation: The following formulas are used in the colorimetric determinations:

$$S \times \frac{Rs}{Rx} \times 500 = \text{milligrams of iron per hundred cubic centimeters of blood.}$$

S = Strength of the standard Rs = reading of the standard

Rx = reading of the unknown.

The iron content of the venous blood of 48 relatively normal children ranged from 40 to 52 mg. per hundred cubic centimeters. The average was 43.9 mg.

In 87 children with various pathologic conditions, the blood iron content ranged from 18.2 to 74 gm. per hundred cubic centimeters.

No constant relationship was found between the amount of hemoglobin, determined by the Sahli and Newcomer methods, and the blood iron concentration. In 88 per cent of 135 cases the amount of hemoglobin determined by the Newcomer method exceeded that calculated from the blood iron content.

AGRANULOCYTIC ANGINA, A Study Based on 18 Cases With 9 Necropsies, Fitz-Hugh, Jr., T., and Comroe, B. I. Am. J. M. Sc. 185: 552, 1933.

The significant data of 18 cases of agranulocytic angina (pernicious leukopenia) are reported with 9 necropsies. All but 4 are dead (78 per cent mortality). Of these 4 only 2 can be considered cured.

The most promising treatment, aside from blood transfusions, seems to be pentose nucleotid (Jackson) although the author's experience with this substance has not been favorable. It seems more definitely to be useless in chronic cases of the disease.

Necropsy study has shown in more than half the cases thus examined a plentiful supply in the leukopoietic centers of the progenitors of the blood leukocytes. This virtual hyperplasia is in marked contrast to the profound peripheral leukopenia characteristic of the disease, and strengthens the previous suggestion of an hypothesis of primary "maturation arrest," rather than primary "aplasia" to account for the hematologic phenomena of the disease.

Agranulocytic angina (pernicious leukopenia) seems to be a disease entity, although it is so closely simulated by certain other conditions that its nosologic status may remain debatable until a potent specific therapy is available, or some other pathognomonic feature is discovered.

DISTILLED WATER, Triply, The Fetish of, Elser, W. J., and Stillman, R. G. J. A. M. A. 100: 1326, 1933.

Over a period of four years and three months, no chill has followed the introduction of physiologic solution of sodium chloride into the veins of patients at the New York Hospital. This solution was made with New York City tap water freshly distilled once, in a Barnstead still run by steam and operated during a large part of the period at the full capacity of the apparatus. This experience demonstrates with certainty that singly distilled water can be used with safety in the preparation of solutions for intravenous administration and that "triply distilled water" is truly a "fetish" in that it is a "material object regarded with awe, as having mysterious powers residing in it . . . . and from which supernatural aid is to be expected."

CONCLUSIONS

The use of triply distilled water in the preparation of solutions for intravenous administration is not necessary.

The use of freshly (and properly) distilled water in the preparation of solutions for intravenous administration is essential.

Solutions introduced directly into the circulation, other than physiologic solution of sodium chloride, should be administered at a rate not to exceed 5 c.c. per minute.

POLYCYTHEMIA VERA, Treatment of With Solution of Potassium Arsenite, Forkner, C. E., Scott, J. F. McN., and Wu, S. C. Arch. Int. Med. 51: 616, 1933.

Six patients with polycythemia vera have been treated by the oral administration of relatively large doses of solution of potassium arsenite.

Distinct improvement in the clinical and hematologic manifestations has occurred in each patient in from twenty to fifty days.

The induced remissions were characterized by reduction of the erythrocyte, hemoglobin and hematocrit values to normal or nearly normal, reduction of the basal metabolic rate to normal, increase in body weight, increase in strength and subsidence or disappearance of symptoms.

The remissions may be prolonged at least for several months by continuation of the medication in reduced amounts.

The method of administration of the drug and the untoward effects due to treatment with arsenic are discussed.

The administration of solution of potassium arsenite to patients suffering from polycythemia vera constitutes a safe and reliable method for the palliative treatment of this disease.

TOE RINGWORM: Effect of Chlorinated Swimming Pool Water on, Spring, D. Am. J. M. Sc. **185**: 775, 1933.

Ringworm of the toes is such a problem among students and gymnasium habitues in general that a possible fungicidal rôle of chlorinated water in swimming pools merits testing. With 6 women swimmers as test objects, it appeared that:

1. It is possible for detached particles of ringworm tissue to retain viable fungi in chlorinated swimming pool water for one and one-half hours. The fungi, however, may be destroyed in two hours.

2. Chlorinated water in swimming pools does not effectually reach and kill fungus between the toes during an average thirty-minute swimming period.

TUBERCULOSIS, Diagnosis of Early: Value of Monocytic Lymphocytic Index, Sullivan, M., and Jones, P. H. Am. J. M. Sc. **185**: 762, 1933.

$$\text{Index} = \text{ratio of monocytes to lymphocytes} = \frac{L}{M}$$

$$\text{Average normal index} = 2.9$$

It has been demonstrated that the monocyte plays an important rôle in tuberculosis. An increase in the number of monocytes of the circulating blood is indicative of activity. A study by the Sabin technic of the number of monocytes and relative proportion of monocytes to that of lymphocytes, that is, the monocytic-lymphocytic ratio, often proves of invaluable assistance in diagnosis. In active tuberculosis the monocytes are markedly increased, and there is a reversal of the monocytic-lymphocytic index. In arrested tuberculosis there is an increase in the lymphocytes, with the total number of monocytes increased but less in number than the total lymphocytes. It has also been shown that there is a storehouse for monocytes in the tuberculous subject, which does not exist in the normal nontuberculous subject. If the total number of monocytes and the monocytic-lymphocytic ratio is determined before and after the administration of tuberculin subcutaneously, the blood of patients in which a definite focal reaction is obtained will show a marked increase in the number of monocytes and a shift in the monocyte-lymphocyte ratio. This simultaneous provocation of focal reaction and increase in circulating monocytes is strong evidence of the presence of tuberculosis.

BONE MARROW Biopsy, Custer, R. P. Am. J. M. Sc. **185**: 617, 1933.

Using rigid aseptic precautions, the skin and subcutaneous tissue are infiltrated at least 1.5 cm. to right and left of the midline with a 2 per cent solution of novocain for a distance of 3 cm. above and below the attachment of the fourth rib. After a lapse of five minutes a sagittal incision, 4 cm. in length, is made down to, but not including, periosteum. The needle is inserted beneath the periosteum and the subperiosteal space is infiltrated with novocain over the operative field; thus all sensitive parts have been rendered anesthetic. The periosteum is incised in the line of skin incision and elevated, exposing an area slightly greater than 1 cm. in diameter. Using an ordinary trephine of 1 cm. diameter, the ventral table of the sternum is cut; the marrow cavity has been entered when the sensation of a slight downward thrust is experienced (care must be exerted not to continue drilling through dorsal table into mediastinum). The trephine is tilted slightly back and forth in sagittal and transverse planes, thus breaking the fine trabeculae of the underlying cancellous bone. The trephine is withdrawn; the bone button will come away with the drill or can be removed with tissue forceps. The margin and floor of the myeloid cavity are scraped with a sharp bone curette; the curettings and button are handed to the pathologist (whose presence at operation is imperative) on a sterile sponge. The cavity is packed firmly with gauze until all oozing has ceased; the

periosteum is replaced over the bone defect, but not sutured; skin and subcutaneous tissue are approximated with interrupted No. 00 plain catgut (or horsehair) sutures (in female subjects a carefully laid subcuticular suture may be preferable). Sterile dressings are applied and the patient returned to bed; dressings may be discontinued after the sutures have been absorbed (usually about the sixth day). There is eventual fibrous or osseous replacement of the bone defect and the resulting scar an inconspicuous, fine, white line.

Microscopic Technic: A tiny fragment of the curettings should be teased out and stained supravitally.

The button and remaining curettings are fixed promptly in Zenker-formal solution for four to six hours, washed for an equivalent length of time in running water and decalcified in a solution of equal parts of 85 per cent formic acid and 20 per cent sodium citrate (six hours is usually sufficient, although the tissue may be left overnight without destroying the staining quality of the cells). The tissue is washed in running water for about four hours, transferred through 95 per cent alcohol (twelve hours), absolute alcohol (six hours), chloroform (45 minutes), chloroform and paraffin (thirty minutes in oven), paraffin (40 to 42° C.) (fifteen to twenty minutes), paraffin (53 to 56° C.) (2 changes totaling thirty minutes) and finally embedded in paraffin, being careful to embed the button with the marrow surface downward. The tissue is blocked and cut in the usual fashion, preferably not over 5 micra in thickness, mounted on albuminized slides and placed in the oven for at least two hours (better overnight to insure flat sections). Paraffin is removed by 2 changes of xylol, following with the slides are transferred through the following solutions: 95 per cent alcohol, 95 per cent alcohol and iodine (cherry red solution) until the mercury of the fixative is removed, 95 70, 50 and 30 per cent alcohol and finally through 3 changes of distilled water to insure removal of all alcohol. Slides are then placed in the stain mixture prepared as follows:

Solution I

Eosin fur Blut (Grubler)	1 gm.
Distilled water	1 liter.

Solution II

Azure II	1 gm.
Distilled water	1 liter.

(Azure II as supplied by National Aniline and Chemical Company only.)

Mix solutions as follows in the indicated order:

1. Distilled water	80 c.c.
2. Solution I	20 c.c.
3. Solution II	10 c.c.

Stain from late afternoon until the following morning. Differentiate, controlling differentiation under the microscope.

1. Alcohol, 95 per cent, until blue stain ceases to come off in a cloud.

2. Alcohol, 95 per cent, until differentiation is complete (bone trabeculae, red blood cells, eosinophil granules are the landmarks and should be red; nuclei should be sharp and brilliant blue).

3. Transfer rapidly through 2 changes of absolute alcohol for dehydration.

4. Xylol (3 changes) until clear. At this time check stain under high dry power; if underdifferentiated, slide can be carried back into alcohol; if overdifferentiated, it is practically useless. Decolorizing in acid-alcohol and restaining are possible but results do not justify the effort; better cut new sections.

LIVER FUNCTION, A New Test of: The Synthesis of Hippuric Acid, Quick, A. J. Am. J. M. Sc. **185:** 630, 1933.

The Test.—Five and nine-tenths grams of sodium benzoate dissolved in 30 c.c. of water is administered one hour after a breakfast consisting of coffee and toast. The

patient is then given ½ glass of water. Immediately after taking the drug the patient voids, and then collects complete hourly specimens for four hours. These are preserved with toluene, and hippuric acid determined in each specimen. In normal adults the output of benzoic acid as hippuric acid is approximately 1 gm. or more during the second and third hours, and the total for the four hours is from 3 to 3.5 gm.

For clinical purposes a simple method has been developed which will be described as Method 1.

Method 1.—Simpler Clinical Method. Each hour specimen is measured, transferred to a small beaker and acidified with concentrated hydrochloric acid until acid to Congo red; 1 c.c. of the acid is usually sufficient. The solution is vigorously stirred until the precipitation of the hippuric acid is complete, and then is allowed to stand for one hour at room temperature. The precipitate is filtered off on a small Buchner funnel or a filter plate, washed with a small quantity of cold water and allowed to air dry. The hippuric acid thus obtained is either weighed (to the second decimal place, which is sufficiently accurate) or titrated with 0.2 N sodium hydroxide, using phenolphthalein as indicator. To obtain the total hippuric acid, one must add to the amount thus obtained the calculated quantity remaining in solution; 100 c.c. of urine will dissolve 0.33 gm. of hippuric acid. In case any specimen exceeds 125 c.c. it should be slightly acidified with acetic acid and concentrated on the water bath to about 50 c.c. before precipitating the hippuric acid. The results are best expressed in terms of benzoic acid. To convert hippuric acid to benzoic acid, one multiplies by 0.68.

Method 2.—This determination depends on the isolation of hippuric acid from the urine by extraction with ether, and subsequent hydrolysis by means of hydrochloric acid. The glycin thus liberated is determined by the well-known formol titration: 5 to 10 c.c. of urine are transferred to the extraction tube of a continuous extractor, 1 c.c of 5 N sulphuric acid and 2 drops of 10 per cent sodium tungstate added. The sample is extracted with ether for ninety minutes. The ether is removed by distillation, 10 c.c. of 20 per cent hydrochloric acid is added to the crystallin residue, and the flask is connected with an air condenser. The solution is refluxed on a hot plate for one hour, and then is transferred to a small dish, and evaporated to dryness on a water bath. The residue is dissolved in about 20 c.c. of hot water, a small amount of decolorizing charcoal (norit) added, and the solution filtered. The residue is washed several times with hot water. One drop of 1 per cent neutral red indicator is added to the filtrate, which is then neutralized to P_H 7. At this P_H neutral red is slightly pink, i.e., midway between yellow and red. Six drops of 1 per cent phenolphthalein and 10 c.c. of neutralized 40 per cent formaldehyde are added, and the solution titrated with 0.1 N sodium hydroxide until the deep red color matches the standard, which is prepared by mixing 20 c.c. of distilled water, 10 c.c. of neutralized formaldehyde, 6 drops of phenolphthalein, 1 drop of neutral red and 0.3. c.c. of 0.1 N sodium hydroxide. Since 1 c.c. of 0.1 N sodium hydroxide is equivalent to 1 c.c. of 1 N glycin, the titration value, corrected for the 0.3 c.c. blank, can be converted directly either to hippuric or benzoic acid.

Diminished excretion of hippuric acid was observed in luetic cirrhosis, catarrhal jaundice and in obstructive jaundice of moderately long standing. Normal results were obtained in cholecystitis and in 2 cases of portal cirrhosis.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Practical Hematological Diagnosis*

THIS book, both because of the comprehensive survey of its subject matter and especially because of the very practical manner in which it is covered, should prove of especial value to the protecting physician from whom it deserves, a generous reception.

So many and diverse have been the developments in the laboratory study of the blood that it is sometimes a matter of difficulty to evaluate their results and significance.

In this volume Drs. Pepper and Farley have presented in an eminently clear and utilizable manner the more salient facts concerned with the cytological examinations of the blood. Although methods for the more commonly used procedures are described in clear detail; the book is not a laboratory manual but in greater part is devoted to a discussion of the significance of blood findings and their clinical application to the study of disease.

The book is divided into three parts, the first of which describes the components of the blood in accordance with their hematological interest, selected methods for their study, and the practical significance of their determination.

Part II discusses in an orderly, detailed, and satisfactory manner the diseases of the hematopoietic system and the hematological findings applicable to their diagnosis and study; while Part III discusses the hematology of diseases not primarily of the blood.

A section of great interest and great value to the practitioner is that in which is described the outstanding features of the blood picture in nearly 400 diseases and conditions alphabetically arranged so that the information is readily accessible.

An extensive bibliographical index (ten pages) testifies to the thoroughness with which the literature has been covered and the reputation of the authors assures an authoritative presentation.

This volume should find a wide field of usefulness.

Arteriosclerosis†

THAT a man is as old as his arteries, and that arteriosclerosis is the inevitable and, as it were, normal concomitant and aftermath of the process of aging have long been more or less generally accepted axioms.

Like many another in the history of medicine these, too, have given way to the concept that arteries, perhaps, are only as old as their environment, a concept suggested by the absence of arteriosclerosis in the presence of extreme old age and its occasional postmortem demonstration in children.

It is, of course, natural that a condition so commonly encountered and responsible for such widespread secondary lesions and important functional failures should long have attracted the interest of investigators. Unfortunately, however, morphologic studies have added but little to the understanding of the problem and, indeed, may now be said to have reached an impasse. It has become increasingly evident, not only that degenerative lesions constitute

*Practical Hematological Diagnosis. By O. H. Perry Pepper, M.D., Professor of Clinical Medicine, Univ. of Pa., and David L. Farley, M.D., Associate in Medicine, Univ. of Pa., 562 pages; 3 colored plates; Cloth, Philadelphia, Pa., W. B. Saunders Co.

†Arteriosclerosis, A Survey of the Problem. Edited by Edmund V. Cowdry. Cloth, pp. 617, 58 figures. New York. The Macmillan Co.

one of the most important of problems in medicine and human economy, but also, that new avenues of approach to their study must be formulated which will take into account the advances of biochemistry and the concept that arteries and the cardiovascular system in general are living things and maintain a dynamic relationship to other organs.

With these thoughts in mind, under the auspices of the Josiah Macy Jr. Foundation, a thorough survey of the problem of arteriosclerosis as a whole was undertaken, the results of which are presented in this volume edited by E. V. Cowdry of Washington University.

The purpose of the survey was threefold:

(1) To determine the data concerning arteriosclerosis which can be regarded as established and their relation to one another; (2) To discover and to definitely formulate the principal problems awaiting solution; and, (3) To suggest the best means of attacking them.

The volume properly starts with an historical résumé by E. R. Long of the development of our knowledge of arteriosclerosis, followed by an account of the structure and physiology of blood vessels (E. V. Cowdry), and the physical properties of blood vessels in health and disease (C. Bromwell).

The technic of microincineration for the study of the mineral constituents of blood vessels is then described by its originator (A. Policard).

The statistical data of arteriosclerosis is surveyed by E. Sydenstricker and the occurrence of arteriosclerosis in lower mammals and birds and its relation to the disease in man discussed by H. Fox.

Having thus outlined the broader aspects of the problem, as it were, the remaining chapters are concerned with experimental aspects and cover: Race and Climate as Possible Factors (P. Stocks); Nutrition in Relation to Arteriosclerosis (S. Weiss and G. R. Minot); Pathogenesis (W. Ophuls); Experimental Arteriosclerosis in Animals (N. Anitschkow); The Chemistry of Arteriosclerosis (H. G. Wells); Acute and Chronic Infections as Etiological Factors (W. G. MacCallum); Retinal Arteriosclerosis (J. S. Friedenwald); Arteriosclerosis of the Brain and Spinal Cord (S. Cobb and D. Blain); Coronary Arteriosclerosis (H. Karsner); Sclerosis of the Pulmonary Arteries (H. Karsner); Arteriosclerosis of the Abdominal Viscera and Extremities (E. T. Bell); Hypertension in Relation to Arteriosclerosis (F. Lange); Hereditary Aspects of Arterial Hypertension in Relation to Arteriosclerosis (G. D. Williams); The Treatment of Arteriosclerosis (J. H. Wyckoff); and a final Summary by A. E. Cohn.

An author index and subject index complete the volume.

It can be said with confidence that this volume constitutes the most complete, the most authoritative, and the most illuminating and outstanding contribution to the study of arteriosclerosis yet made. Student, investigator, pathologist, physician at large, all can read it with profit. Without doubt it will remain an outstanding reference work for some time to come.

In format and general workmanship the volume leaves nothing to be desired.

The Elements of Medical Treatment*

THIS book represents the subject matter of a lecture course on elementary therapeutics and has for its purpose the presentation of basic principles and their application to the commoner diseases met with in practice with special attention to the prescription of drugs.

Eminently practical in purpose, it is equally practical in the handling of the subject and may be recommended with confidence to the physician as well as the student. Those who master the contents of its twenty-four chapters will never be at loss when confronted with the usual exigencies of medical practice in general.

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*The Elements of Medical Treatment. By Robert Hutchinson, M.D., Physician to the London Hospital. Cloth, p. 188, Baltimore, William Wood and Co.

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EDITORIAL

Divinyl Oxide in the Twilight Zone

ONE of the greatest chapters in human history is that which deals with the discovery and use of anesthesia. In comparison with this, how pale and insignificant to us appear such incidents as the ascent of Moses up into the mountain to meet Jehovah face to face, the flight of Elijah up to Heaven in a chariot of fire, or the conquering of the world by Alexander the great. And it may not be too much to surmise that the medical historian of a few generations hence may also add to this list the fall of the Roman Empire, the crucifixion, the rise of the papacy and the world war. For anesthesia will spread its wings like a conquering angel over pain so long as man shall suffer.

Like most things in this life the anesthesia of today is not yet perfect. There is still ample room for improvement. And, thanks to a numerous company of eager workers, our conceptions, our ideals, and our accomplishments in anesthesia are constantly advancing toward a happier and more effective goal.

And now like the flitting fragrance of some new fruit from California come whispering echoes of a new anesthetic still lingering in the offing at San Francisco. In that city, where Dr. C. D. Leake[1] and his colleagues work, and in Edmonton, Canada, where Drs. Gelfan and Bell[2] have studied this compound, it is known as divinyl ether, or divinyl oxide. But it seems probable that we may presently hear much more of this preparation under another name (vinesthene).[3] Vague opinions, good, bad and indifferent, regarding this compound have reached the writer. And it is obvious, that, for the moment, the drug must appear to pharmacologists as a hopeful vision in the twilight. It is a little too early yet to determine whether this is the twilight of the evening, or that which precedes the coming dawn. But it appears reasonable to believe that enough good work has now been done to indicate that this preparation promises something, and perhaps a great deal, for medicine in the future.

The drug is about as explosive and inflammable as ether, and on exposure to light and air it tends to polymerize with the formation of a gel and later a resin. This latter reaction is hastened by acids but may be prevented by faint traces of alkali. Major has also observed that partial decomposition may lead to the formation of some formaldehyde and formic acid. The drug is more volatile than ether, it boils at 23.8° C. and its specific gravity is 0.77. It decolorizes potassium permanganate solutions and this reaction may lead to its detection in various biologic fluids, and even urine, in which some of the drug appears to be excreted although the greater quantity seems to be rapidly eliminated by the lungs.

Only chemically pure samples should be used for anesthesia, since those which have undergone slight decomposition and contain small amounts of formaldehyde and formic acid are irritating and dangerous. This latter feature may serve as a useful guide in the detection of deteriorated samples, and it may be added that such samples also tend to become cloudy in addition to acquiring a stinging odor. Samples improperly made or not sufficiently purified may contain chlorinated ethers as impurities, as the drug is made from chlorinated ethyl ether.

The preparation volatilizes readily, and when given by the open drop method it is absorbed by the lungs quickly and loss of consciousness and relaxation may be attained much earlier than with ethyl ether. The drug has a strong sweetish taste but is less irritating than ether and leaves no biting or burning sensation after inhalation. When pure its sweetish, ethereal odor is not so pungent and disgusting as that of ethyl ether. A new method of preparing the compound has recently been developed by Ruigh and Major,[4] and it appears that this process yields a practically pure compound. It seems highly probable that use of slightly deteriorated samples would lead to respiratory complications. But first class samples are less irritating, less nauseating and less stormy and lasting in their action than is ethyl ether. There is less salivation, induction is usually quicker and smoother, and postanesthetic nausea and vomiting are apparently less than with ethyl ether. The minimal certain anesthetic concentration for mice is 3.9 volumes per cent, while the minimal certain anesthetic concentration of ethyl ether is 4.7 volumes per cent.

With twice or more of the above respective concentrations danger appears in about the same degree with the use of each drug. It may be noted that with the minimal effective concentration of each, anesthesia appears quicker with divinyl oxide than with chloroform. Respiratory failure is the immediate cause of death in fatal cases under divinyl oxide, and in these the heart and circulation appear to be in very good condition at the time of respiratory cessation. Apparently resuscitation may be effected more easily and more certainly than after comparable degrees of poisoning with ethyl ether. If administered with deficient oxygen divinyl oxide may cause a slight rise in the hydrogen ion concentration and sugar content of the blood. With sufficient oxygen this does not occur.

Dogs anesthetized for periods of thirty minutes daily for ten days with no anoxemia showed practically no pathologic changes in the liver, heart, or other internal organs with the possible exception of some slight effect on the kidneys and urine secretion. With anoxemia the kidneys seem to be the organs more likely to undergo pathologic changes. This may be due to some excretion of the drug by the kidneys. The liver seems free from any such changes as might be produced by chloroform.

Dr. Gelfan[2] reports anesthetization of himself twice for periods of ten and eighteen minutes respectively with divinyl oxide, while Dr. Winifred Hughes has been anesthetized with the drug for five minutes. On each of these occasions the subjects recovered and were able to converse rationally and to get up and walk about the room with full coordination in about two minutes after administration of the drug was stopped. No after-effects whatever were noted. This amount of clinical evidence is, of course, pathetically small, but it merely illustrates the extreme difficulty which one (and particularly a laboratory worker) may have in getting his discoveries tried out clinically. And this is the more pathetic when one reflects on the actual status of medical practice in general, and of surgery in particular, in the world today.

The formula for divinyl oxide is $CH_2.CH.O.CH.CH_2$, and on the basis of its chemical similarity to both diethyl ether and ethylene Dr. Leake[5] predicted that it should possess comparable pharmacologic properties. It seems from all the evidence at hand that the drug bids fair to fulfill this prediction. In the meantime others look on with mingled feelings of doubt and hope, and perhaps with a typically racial complex involving in the very faintest degree a few other historically human sentiments, while three or four workers shoulder the responsibility of hustling this drug off to an ignominious oblivion, or of raising it to a position of respect or even of high honor in the surgical armamentarium of the immediately approaching future.

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D. E. J.